



Effect of paper industry effluent on enzyme activity and protein profiling of Chickpea (*Cicer arietinum* L.)

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Received: November 8, 2016; Revised received: May 5, 2017; Accepted: September 7, 2017

Abstract: Chickpea (*Cicer arietinum* L.) is a legume which is mostly cultivated in India than other countries that can give significant amounts of dietary minerals and protein to humans. The effect of paper industrial effluent on chickpea (*C. arietinum* L.) were analysed along with different concentration (10%, 20% 40%, 60% 80% and 100%) and pure tap water as a control to compare the effect of paper industrial effluent for one week. The amount of protein were comparable with control, their amount was increased at 40% in effluent treated seeds. The maximum activity of enzymes was found below 40% level of effluent. In this study protein profile of imbibed seeds, shoot, root and residual cotyledons were examined under the different concentration of effluent. Polyacrylamide gel electrophoresis of total protein showed that the maximum number of protein bands seen in the imbibed seeds whereas minimum number of protein bands observed in the root. SDS-PAGE revealed that less degradation and/or more rapid accumulation of proteins occurred in higher molecular weight proteins. From this study, it is clear that the industrial effluent rich in organic matter and plant nutrients are finding their use in agriculture as the cheaper way of disposal.

Keywords: Amylase, Chickpea, Imbibed seeds, Paper industry effluent, SDS-PAGE

INTRODUCTION

Chickpea (*C. arietinum* L.) is a legume crop, which belongs to the family Fabaceae. It is the second most important legume crop after dry beans (Varshney *et al.*, 2012). It is a valuable source of protein, carbohydrate, fibre and many essential vitamins and minerals (Roy *et al.*, 2010). The amount of carbohydrate, protein and reducing sugar were increased under stress condition (Chaudhary *et al.*, 2016). India has the first rank in the world, contributing 68% of the global chickpea production, cultivated in 9.21 million ha, producing a total of 8.22 million tonnes with an average yield of 892.5 kg/ha (FAOSTAT, 2013).

Industrialisation is an important sign for the development of any nation. Today it has become a matter of major concern in the deterioration of the environment; with the rapid growth of industries the industrial waste water has increased tremendously (Amathussalam *et al.*, 2002). Agriculture is the backbone of any economy, nowadays it has been adversely affected by the upsurge in the indiscriminate dumping and disposal of the wastes into land and watercourses. If the excessive amount of these waste are absorbed by plants, the yield may get reduced and the plant may die due to phytotoxicity (Singh and Sakal, 2001). Water resources are

affected by industrial pollution extremely. Pollution caused by industrial effluents is a severe problem throughout the world. Paper industrial effluent that has not been treated properly has an obnoxious odour when discharged into the environment. The effluents not only affect the plant growth but also degrade the soil properties when used for irrigation (Patel *et al.*, 2004). So it is necessary to conduct experiments to find out the impact of these effluents on agricultural crops before they are recommended for irrigation. A laboratory experiment was designed to know the effect of different concentration (0-100%) of paper industrial effluent on protein content, enzyme activity and profile of protein in chickpea using SDS-PAGE.

MATERIALS AND METHODS

Seed selection: Seed of uniform size, shape, colour and weight were selected. Now seed was sterilised with the HgCl₂ solution, washed thoroughly with distilled water and kept in different concentration of industrial effluent separately. Seed simultaneously kept in distilled water, which constituted as a control. After requisite imbibitions of 42 hours, germination was assessed by radical emergence (2-3mm).

Effluent collection and experimental setup: The

effluent sample was collected from paper industry in 5-liter plastic cans and then stored in dark room and diluted in different concentration (10, 20, 40, 60, 80, and 100%). Chickpea seeds were imbibed in different concentration of paper industrial effluent for up to 24 hours and were grown in Petri plates in three replications and irrigated by different concentration of effluent for up to 7 days for germination. Control was irrigated with an equal volume of distilled water. After 7 days, root shoot separated out from each other by cutting for the analysis. Five seedlings were collected from each treatment and were analysed.

Enzyme activity analysis

Assay of α -amylase: the 50mg sample was homogenised in 8ml buffer (0.2 M Tris-Malate Buffer, pH 7.2,) and centrifuged at 5000 rpm for 5 min. The supernatant was taken and make up the volume 8 ml with the buffer. 1ml of enzyme extract and 1ml of the substrate (starch) were added in a test tube and keep at room temperature for 10 min. Add 3ml of quenching reagent (Iodine) and take O.D. at 620nm (Filner and Varner, 1967).

Assay of β -amylase: the 50 mg sample was crushed in 0.05N phosphate buffer, pH 7, centrifuged at 5000 rpm for 5 min. The supernatant was taken and make up the volume 8 ml with the buffer. 100 μ l sample, 2.9 ml of phosphate buffer and 2ml of DNS reagent was added, incubated in boiling water bath for 5min. and take O.D. at 570nm (Bernfeld, 1995).

Assay of acid phosphate: the 1 gm sample was homogenised in 8 ml buffer (acetate buffer, pH 5) and centrifuged at 5000 rpm for 5 min. 50 μ l supernatant and 25 μ l of PNpp was added and then make up the volume 8 ml by 2.925 ml of acetate buffer, and incubate at 300°C for 30 min. 2 ml of NaOH was added and take O.D. at 430nm (Lea, 1990; Prince *et al.*, 1982; Wilson *et al.*, 1996; Sawhney and Randhir, 2007)

Assay of alkaline phosphatase: the 1 gm sample was homogenised in 8ml buffer (1M Tris buffer, pH 7.5) and centrifuged at 5000 rpm for 5 min. the 50 μ l supernatant and 25 μ l of PNpp were added and then make up the volume by 2.925 ml Tris buffer and incubate at 30°C for 30 min. 2ml of NaOH was added and take O.D. at 430nm (Lea, 1990; Prince *et al.*, 1982; Wilson *et al.*, 1996; Sawhney and Randhir, 2007).

Total protein extraction and SDS-PAGE: Total protein was extracted by Lowry *et al.*, (1951). The 50 mg fresh plant sample was crushed with 8ml phosphate buffer and centrifuged at 5000 rpm for 5 min. The 50 μ l supernatant was taken and add 2.45 ml of 0.1N NaOH and 2ml of reagent A, incubated at 37°C for 30 min., add 5ml of reagent B and again incubated at 50°C for 5 min and then O.D. was taken at 750 nm. A standard curve of BSA (Bovine Serum Albumin) was used for quantification of protein. 200 μ l Dye (Bromophenol blue) was added to each microtube, then mixed well

and incubated at room temperature for 15 min.

SDS-PAGE electrophoresis was performed by 12% acrylamide separating gel and 4% acrylamide stacking gel with 0.5 mm spacers. The separating gel contained 6.68 ml of distilled water, 5 ml of 1.5 M Tris-HCl, pH 8.8, 200 μ l of 10% (w/v) sodium dodecyl sulphate (SDS), 8 ml of 30% acrylamide (Acrylamide, Bis-acrylamide ratio 29.2 : 0.8), 10 μ l of TEMED and 100 μ l of 10% (w/v) ammonium persulphate. The stacking gel consisted of 1.2 ml of distilled water, 0.5 ml of 0.5 M Tris-HCl, pH 6.8, 20 μ l of 10% (w/v) SDS, 267 μ l of 30% acrylamide, 2 μ l of TEMED and 10 μ l of 10% (w/v) ammonium persulphate.

10 μ g of protein was mixed in a microtube with 1/20 volume of β - mercaptoethanol in a final volume of 10 μ l. After incubation at 95°C for 5 min and a brief cooling on ice, 10 μ l was loaded per lane and electrophoresed at 50 mA for 3-4 hours with 1X SDS-PAGE running buffer (100 mM glycine, 25 mM Tris and 0.1% (w/v) SDS). The gels were stained with Coomassie Brilliant Blue R-250 solution [40% (v/v) methanol, 10% (v/v) acetic acid and 0.25% (w/v) Coomassie Brilliant Blue R-250] and rocked at room temperature for 30 min. These gels were destained [in 40% (v/v) methanol, 10% (v/v) acetic acid and 3% (v/v) glycerol] at room temperature. Gel image was taken.

RESULTS AND DISCUSSION

The α -amylase is an important enzyme plays an important role in the development of different parts of the plant by breaking stored starch molecules into glucose molecules. In imbibed seeds (IS), α -amylase was shown maximum activity at 40% concentration of effluent in comparison to control. In shoot and residual cotyledons (RC), the maximum α -amylase activity was observed at 40% concentration of effluent, while in root no effect was found (Fig. 1). The similar results also reported by Divyapriya *et al.*, (2014). α -amylase activity were diminished progressively with increasing concentration of effluent (Chugh and Sawhney, 1996; Sang *et al.*, 1997). The present investigation showed that, complete inhibition of seed germination above 80% effluent concentrations was correlated with less water uptake which was insufficient for triggering α -amylase enzyme in seeds. All these changes (physiological and biochemical) in the seeds was observed due to the cumulative effect of excess amount of TDS, pH, BOD and COD in the effluent (Chaudhary *et al.*, 2016). This observation has the conformity with the result obtained Kumari *et al.*, (2014) who reported that the germination of *Vigna radiata*, *Oryza sativa*, *Acaiaholosericca*, and *Leucaena leucocephala* was totally inhibited at 75% and 100% tannery effluent. The β -amylase activity decreased with increase the concentration of industrial effluent (Fig.2). In IS, β -amylase activity increased with increasing the concentration of effluent and maximum activity was observed at 80%

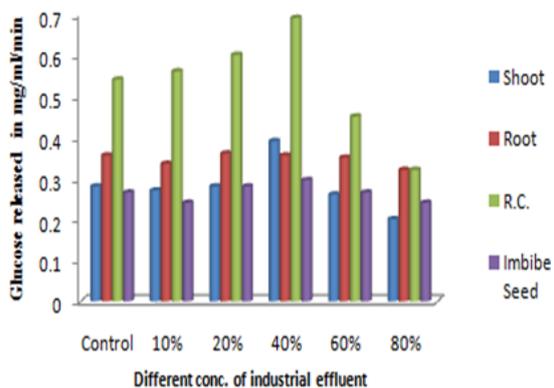


Fig. 1. Effect different concentration of industrial effluent on alpha amylase activity in germinating seeds after 7days and imbibed of *Cicer arietinum* (cv. C-235).

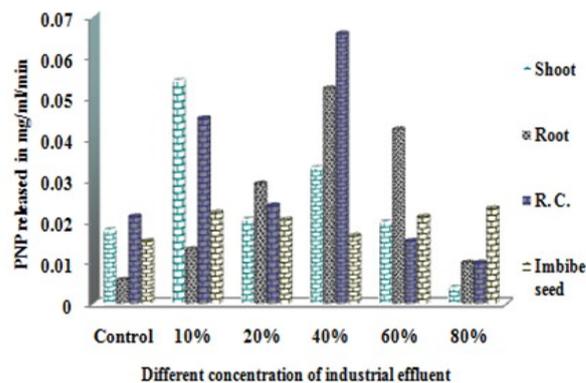


Fig. 3. Effect of the different concentration of industrial effluent on Acid phosphatase in imbibed seeds and seedling

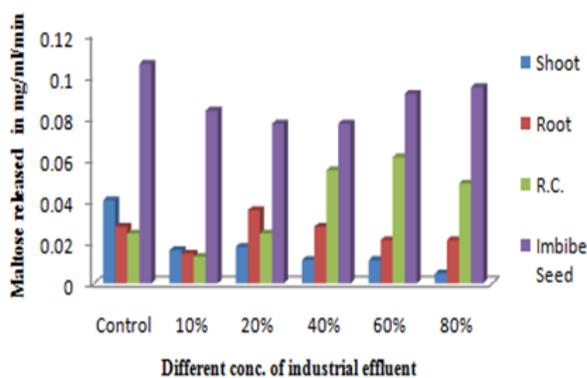


Fig. 2. Effect of different concentration of industrial effluent on Beta amylase activity in germinating seeds after 7days and imbibed seeds of *Cicer arietinum* (cv. C-235).

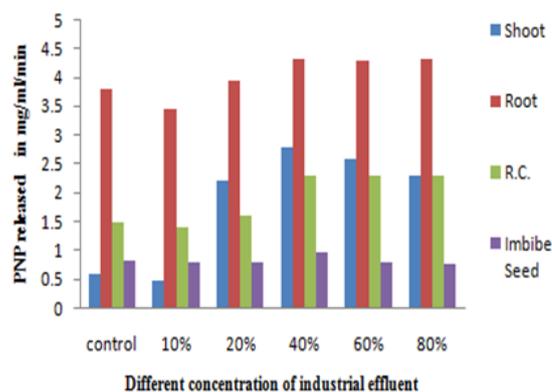


Fig. 4. Effect of different concentration of industrial effluent on Alkaline phosphatase in imbibed seeds and seedling parts of *Cicer arietinum* (cv. C-235).

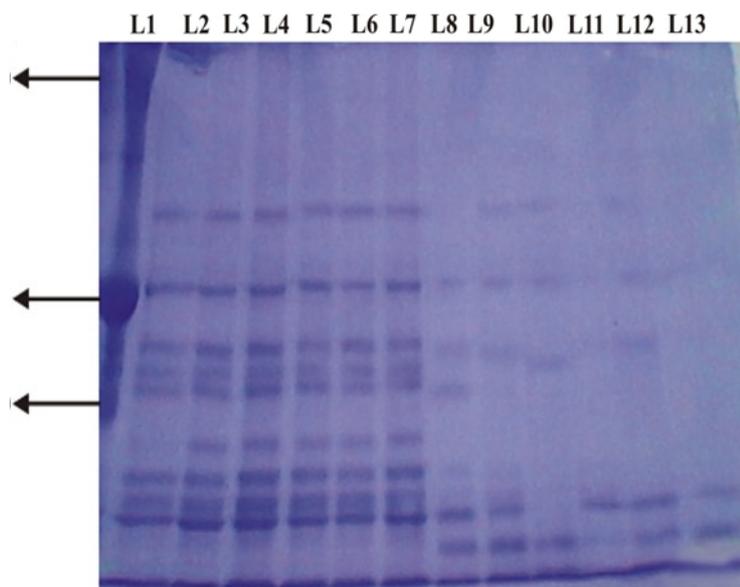


Fig. 5. Protein profile of *Cicer arietinum* imbibed seeds (IS) and residual cotyledon (RC) under control and treated (by paper industrial effluent) on 12% SDS-PAGE [lane: 1- marker, lane: 2- control, lane: 3- IS (10%),lane: 4- IS (20%),lane: 5- IS (40%),lane: 6- IS (60%),lane: 7- IS (80%),lane: 8- RC control, lane: 9- RC (10%),lane: 10- RC (20%),lane: 11- RC

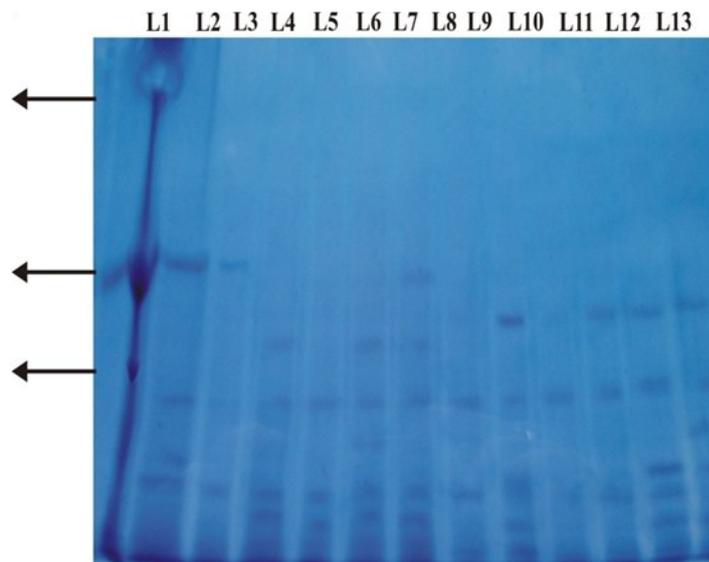


Fig. 6. Protein profile of *Cicer arietinum* root (R) and shoot (S) under control and treated (by paper industrial effluent) on 12% SDS-PAGE [lane: 1- marker, lane: 2- control, lane: 3- R (10%), lane: 4- R (20%), lane: 5- R (40%), lane: 6- R (60%), lane: 7- R (80%), lane: 8- S control, lane: 9- S (10%), lane: 10- S (20%), lane: 11- S (40%), lane: 12- S (60%), lane: 13- S (80%)].

concentration but less than control. In shoot and root, the maximum activity of β -amylase was observed at 20% concentration of effluent but at other doses negligible effect was observed. While β -amylase activity was increased with increasing the concentration of effluent and maximum activity was observed at 60% concentration in RC. The similar result also reported by Upadhyay and Pandey, (2013), as they investigated that the total amylase of wheat, maize and sweet pea was decreased under copper stress. The β -amylase activity was significantly decreased by increasing the concentration of effluent (Nath *et al.*, 2005; Nath *et al.*, 2009).

In IS, maximum activity was observed at 80% concentration and minimum at 40% concentration of effluent but that was less than control. While in the shoot, acid phosphatase activity was observed maximum at 10% concentration and minimum at 80% concentration of effluent in Fig. 3. But in roots and RC acid phosphatase activity was found maximum at 40% concentration and minimum at 80% concentration of effluent. Acid phosphatases have various metabolic functions but in adverse condition, enzyme activity was inhibited. So that plant in control condition had maximum activity (McLachlan, 1980; Duff *et al.*, 1994). In IS no effect on enzyme activity was observed. In root, shoot and RC, the alkaline phosphatase (optimum pH 8.0) activity was increased by increasing the concentration of effluent and maximum and minimum enzyme activity was found at 40% and 10% concentration of effluent respectively but less than control represented in Fig. 4. Alkaline phosphatase activity was decreased due to fluoride sensitivity at higher concentration of effluent (Kieleczawa *et al.*, 1992). Acid phosphatase and alkaline phosphatase are phospho-hydrolytic en-

zymes in supplying inorganic phosphate that is required for the maintenance of various cellular metabolisms such as the conversion of starch to sugar (McLachlan, 1980; Agoreyo, 2010). Maximum activity of enzymes was observed below 40% concentration of paper industrial effluent.

SDS-PAGE analysis: All the treatments of chickpea differ in the intensity of band as well as in a number of protein bands. The maximum number of bands was found in IS followed by RC, while root had less number of bands in comparison to shoot. In Fig. 5 three new proteins less than 45 KDa were expressed in IS, while the intensity of bands increased with increasing the concentration of effluent as compared to control. A band of 60 KDa proteins was present in control and treatments with different intensity and intensity of bands decreased in between 90-60 KDa with increasing the concentration of effluent. Two new bands were found less than 45 KDa in RC and the intensity of bands maximum in control and bands intensity decreased as the concentration of effluent increased (Singh and Singh, 2015). Bands of molecular weight greater than 45 KDa are present in control and absent in treatments. On the other hand, low-intensity bands less than 60 KDa was expressed in control but as the concentration of effluent increased no band was observed. Seed storage protein profiles could be useful markers in the studies of genetic diversity (Hameed, *et al.*, 2009 and Kakaei, *et al.*, 2012).

Fig. 6 represents the comparative SDS-PAGE protein profiles of total proteins in chickpea under control and treatments. The highest variation in the electrophoretic profile of shoot and root protein was observed around 60 and 60-45 KDa range proteins. Most of the protein was ranged from less than 60 KDa. In the shoot, bands

of molecular weight greater than 45 KDa are present in treatments and absent in control. New proteins were synthesised in stressed plants (Vitex and Chickpea) for their adaptation in the stressed conditions (John *et al.*, 2011; Kaur *et al.*, 2014) and maximum intensity of band was observed at 10% concentration and decreased with increasing the concentration of effluent (Singh and Singh, 2015). Bands of molecular weight less than 45 KDa are present in treatments and absent in control. This suggests that the earlier expression of this category of proteins may have a role in tolerance response. Absence or presence of some bands may also indicate a functional involvement in stress response. The intensity of few bands remains the same in different concentrations of effluent because the protein content remains the same in all concentrations. Leaves storage protein profiles of *C. arietinum* could be useful markers in the studies of genetic diversity (Kakaei, *et al.*, 2012). But in the root, bands of molecular weight 60 KDa are slightly denser under control condition than treatments and intensity of band decreased with increasing the concentration of effluent. Some new protein bands were expressed less than and greater than 45 KDa, which indicate the functional involvement of the protein in stress condition.

Conclusion

The present study revealed that the paper industrial effluent at lower concentrations (10-40%) promotes the growth and yield of *C. arietinum* but the higher concentrations reduced the same though phytotoxic nature of effluent. Generally, tolerance in paper industrial effluent condition might be due to more rapid accumulation of proteins with higher molecular weight. Hence it was concluded that the number of new proteins was expressed in stressed plants for their adaptation in the stressed conditions. Such kind of proteins could be used as markers in identifying the stressed plants. This investigation is useful to identify the stressed plants. For further analysis using molecular markers will help to understand the genetic variations due to stress.

ACKNOWLEDGEMENTS

Authors are greatly thankful to Dr Manju Chauhan, HOD, D.A.V. (PG) College, Muzaffarnagar for providing necessary lab facilities to complete the work.

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