Response of Mitochondrial ATPase at Seedling Stage of *Triticum aestivum*

Dr. Sushila Sangwan
Dept. of Botany, Government College, Hisar,
e-mail: sushila_sangwan@rediffmail.com

ABSTRACT

Using bioassay system of a crop plant, *Triticum aestivum* crop at early vegetative growth stage, a study was conducted on response analysis of mitochondrial ATPase when crop plant was irrigated refinery effluent. Different dilutions of effluent were made using canal water(Control) Effluent with 75% concentrations resulted in maximum mitochondrial ATPase activity on 10th, 15th, 20th and 25th day after seed germination.

Key Words: Refinery Effluent, Mitochondrial ATPase, Seedling Stage,

DOI Number: 10.48047/NQ.2022.20.12.NQ77732 NeuroQuantology2022; 20(12): 4073-4079

INTRODUCTION

For the present investigation the treated effluent of Panipat refinery was taken for the watering of *Triticum aestivum* crop plant at seedling stage. Panipat refinery is the 7th refinery of Indian Oil Corporation Ltd. It is a technically advanced refinery in the Country and build in an area of around 2200 acres of land. It receives crude oil from Vadinar in Gujarat coast through a 1339 km long pipeline. The refinery is designed for processing both indigenous and imported crude oil. The refinery has processed about 25 types of imported crude oil from countries like Saudi Arabia, Iran, Iraq, Dubai, Kuwait, Malaysia, Nigeria and other African countries since commissioning in 1998. Currently, the capacity of the refinery is being expanded to 12.0M MPTA. Panipat Refinery meets the demands of petroleum products not only of Haryana, but also of the entire north-west region including Punjab, Jammu and Kashmir, Himachal Pradesh, Chandigarh, Uttaranchal state and some parts of Rajasthan and Delhi (Sangwan and Dhankhar., 2010, Sangwan *et.al.,* 2016).

Refinery effluent irrigation could reduce the yield of wheat crops. Wheat crops irrigated with refinery effluent produced significantly less grain than crops that were irrigated with clean canal water. The negative impact of refinery effluent on wheat crops yield / growth is due to the presence of pollutants in the effluent. These pollutants can include petroleum hydrocarbons, organic compounds and heavy metals. These pollutants can damage the cells of wheat seedlings, leading to low vegetative growth and reduced yields (Mengel*et al.*2020, Cabral*et al.et al.* 2020, Konwar*et al.* 2019, Kashem*et al.* 2019, Baruah*et al.*2018, Benavides*et al.* 2015).

Palmgren *et al.* (1990) found that most of the detergents inactivate the ATPase of plasma membrane vesicles from sugar beet leaves in addition to disrupting the permeability barrier to adenosine triphosphate. Ballantyne (1973) observed that *Adenosine triphosphate* production in mitochondria of bean hypocotyls and maize coleoptiles is inhibited by sulphite. Mittal and Dubey (1992) reported that mitochondrial ATPase activity increased in embryoaxes of germinating salt tolerant rice seeds under salinisation, whereas in sensitive cultivars salinity caused decrease in enzyme activity in both endosperms and embryoaxes. Ballantyne and Black (1980) reported that sulphite stimulated ATPase activity in bean hypocotyle mitochondria. Sen and Mukherji (2004) found high ATPase activity in summer when the ATP content was high, indicating an active metabolic period with a high demand for energy and high ATP turnover.

Most of the world's air, water and land resources are now partially poisoned by industrial effluents from industrial processes including those of crude oil and gas. According to a survey, entire country produces 18,422 million litres of waste water per day (Khan, 2000, Hayat*et al* 2021), when the effluent from oil industry are not properly disposed off, there is general belief, backed up by scientific evidences that, they cause pollution to surface and underground water with dangerous consequences to life. The problem of disposal and utilisation of refinery effluent has drawn considerable concern and attention of scientists, technologist and environmental government regulators etc(Sangwan *et.al.*, 2016).

There is a possibility of safe use of this waste water for recycling in industrial process as well as its potential for commercial productivity. If this effluent can be safely utilized for irrigation purpose it will minimize the environmental pollution, reduce the problem related to effluent handling, storage and disposal, preserve the quality of water and reduced the requirement of fertilizer. The treated effluent of almost all of the industries can also be used judiciously for irrigation purposes and hence prevent pollution and disposal problems (Ranganathan *et al.*, 1999; Chhankar *et al.*, 2000; Rao & Rao, 2002,Kashem*et al.*2019,Jamali *et al.* 2017). So, an evaluation of pre-treated refinery effluent impacts on mitochondrial ATPase was done on intense water requiring crop namely wheat crop.

MATERIAL AND METHODS

For Mitochondrial ATPase Quantification, mitochondrial preparation according to Ballantyne (1973) were done. Fifty grams of leaf tissue were taken and grounded in 100 ml of chilled medium consisting of mannitol, 0.3M; tricine, 0.05M; EDTA, 0.001M, $MgCl_2$ 0.0005M; bovine serum albumen, 0.1 percent and cysteine hydrochloride, 0.05 percent and adjusted to pH 7.9. The macerate was squeezed through four layers of cheese cloth. The resulting filtrate was centrifuged at 12,500 rpm for 8 min at 0° C. The pellets were discarded and the superantant was centrifuged again at 30,000 rpm for 12 min. The mitochondrial pellets was suspended in mannitol, 0.3 M; tricine 0.05M; and $MgCl_2$ 0.0005M; and adjusted to pH 7.9.

ATPase activity was determined by Blackmon and Moreland (1971) methods. The ATPase reaction was run at 30° in a mixture of the following in a final vol. of 5 ml: 0.1 M sucrose, 3.0 mM $MgCl_2$, 15 mM KCl, 1.37 mM ATP, 0.1 M tricine (pH7.9). The reaction was initiated by the addition of mitochondrial suspension containing 0.1 mg protein. Aliquots (1ml.) were withdrawn after 10 min and added to 2 ml ice-cold trichloro acetic acid. Inorganic phosphate was determined by Fiske and Subbarao's (1925) method.

All the data obtained were analysed statistically using Sokal and Rohlf (1995). All the data were subjected to mean, standard deviation, correlation and analysis of variance (ANOVA). One-way analysis of variance was applied for determining the significant differences due to effluent applications only while two-way analysis of variance was utilized for test of significance due to the effluent treatments and different time intervals (Sokal and Rohlf, 1995).

RESULTS & DISCUSSION

The effluent water collected from oil refinery, Panipat and canal water(Control) were analyzed for different physicochemical properties and it found that total dissolved solids present in the effluent were 1390.70

mg/L which were higher than that of control water (1450.52 mg/L) but lower than that of the central pollution control board (CPCB, 1995). The effluent was slightly alkaline i.e., pH 7.79. The electrical conductivity of the effluent was lower than the ISI standards. The amounts of inorganic nutrients and heavy metals in effluent were found higher as compared to control water.

Effect of various concentrations of the effluent on mitochondrial ATPase activity at different intervals of time in *Triticum aestivum* was shown in Figure: 1. It was revealed that the mitochondrial ATPase activity increased with the increase in effluent concentration in Dilutions but at 100% concentration, effluent showed reduced the enzyme activity. Effluent with 75% concentrations resulted in maximum mitochondrial ATPase activity at early vegetative growth of *Triticum aestivum* crop plants. On 25th day, the increase of enzyme activity was 0.31% , 0.63% and 1.25% more than the control at 25%, 50% and 75% concentration of the effluent respectively. The higher concentrations of the effluent lowered the enzyme activity by 0.31%.

A high mitochondrial ATPase activity observed in response to low concentration of refinery effluent which resulted in high ATP content, indicating an active metabolic period with a high demand for energy and high ATP turnover. High ATPase activity can be associated with a physiologically active plant. ATPase provide continuous input of free energy for active transport and various bio-synthetic reactions (Dubey et al. 1987).

Possible induction of ATPase due salinity in seedling and at early vegetative growth stage of cultivar might be helpful in maintaining the higher metabolic status of the cells by providing higher rate of phosphate and energy liberation as well as active transport and biosynthetic events. it was observed that salts like k, Cl, Na_2So_4 and $MgSo_4$ present in refinery effluent caused significant increase in enzyme, activity compared to controls when incorporate in the irrigation. Mitochondrial ATPase showed requirement for Mg^{2+} (Blacknon and Moreland, 1971) and presence of ample amount of Mg^{2+} in refinery effluent might be responsible for higher ATPase activity in *Triticum aestivum*.

In contrast to this undiluted refinery effluent showed decrease in Mitochondrial ATPase activity in *Triticum aestivum.* ATPase activities found to be inhibited in vivo due to high salinity present in undiluted refinery effluent. Further move high amount of sucrose inhibits ATPase activity. Similar results were observed by Blackman and Moreland (1971); Eukanson (1987), Mittal and Dubey (1992) and Sen and Mukherji (2004) reported decrease ATPase activity in maize seedling under drought stress.

Sen and Mukherji (2004) reported that in Winter, low phosphorus lead to depressed ATP content which resulted in ATPase being less active indicating poor photosynthetic effeciency, low production of plant food matters and consequently a less demand for energy. Low Pi and ATP content along with low ATPase hampers the yield as well as yield quality.

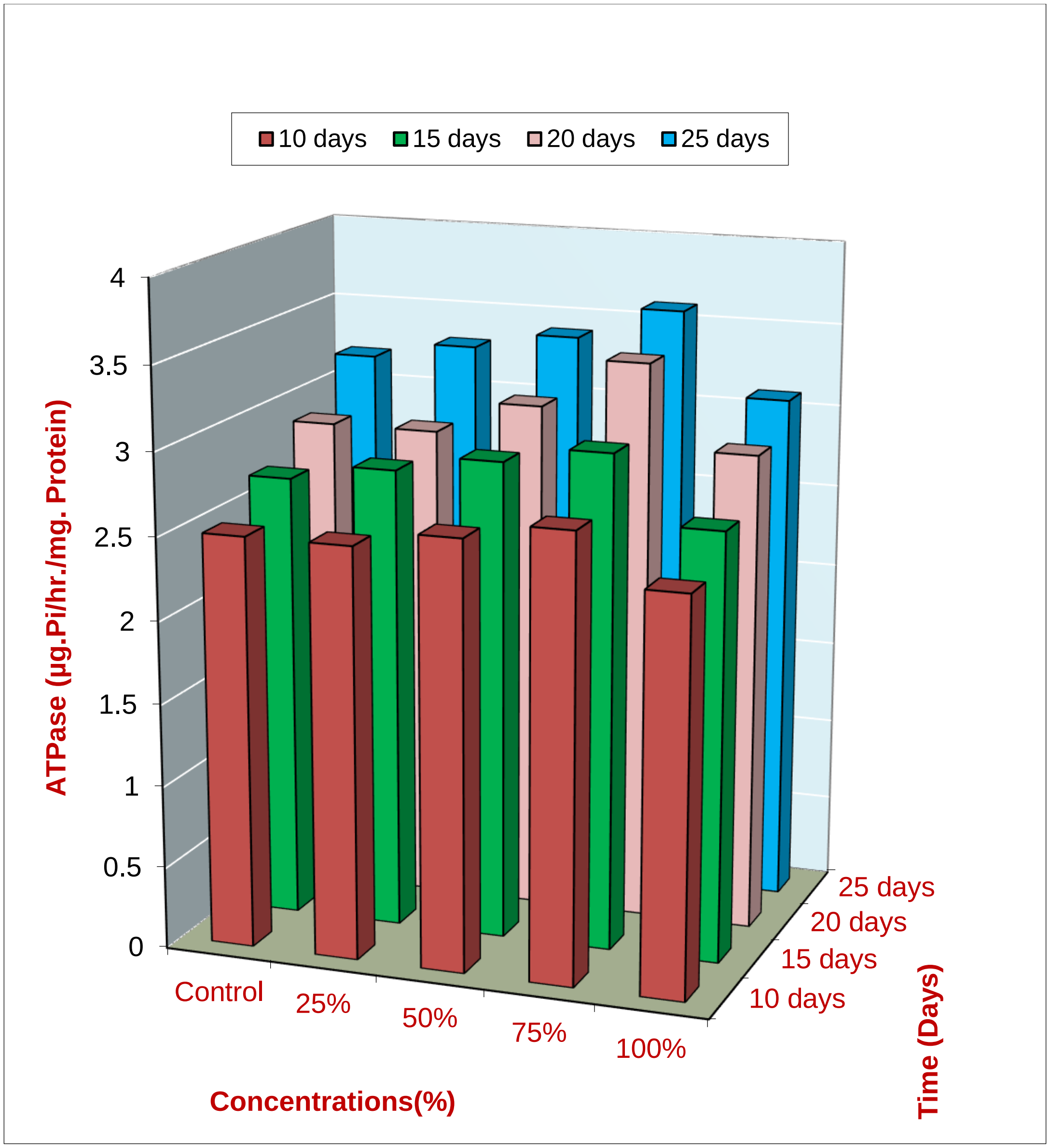

Fig. 1 Temporal variations of mitochondrial ATPase at seedling stage of wheat Plant in response to different concentrations of effluent

Mitochondrial ATPase Anova: Two-Factor Without Replication				
SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	4	11.3	2.825	0.089167
Row 2	4	11.5	2.875	0.109167
Row 3	4	12	3	0.113333
Row 4	4	12.7	3.175	0.1625

Row 5	4	11	2.75	0.096667	
Column 1	5	12.7	2.54	0.013	
Column 2	5	14	2.8	0.025	
Column 3	5	15.2	3.04	0.048	
Column 4	5	16.6	3.32	0.037	
ANOVA					
Source of Variation	*SS*	*df*	*MS*	*F*	*F crit*
Concentration	0.445	4	0.11125	28.40426	3.259167
Time	1.6655	3	0.555167	141.7447	3.490295
Error	0.047	12	0.003917		
Total	2.1575	19			

CONCLUSION

Analysis results of above study clearly indicated the beneficial impacts of the treated refinery effluent up to dilution of 75% on the physiology of early growth stage of wheat crop. A high mitochondrial ATPase activity in the leaves of *Triticum aestivum* at early vegetative growth stage was observed in response to low concentration of refinery effluent which resulted in high ATP content, indicating an active metabolic period with a high demand for energy and high ATP turnover.

AKNOWLEDGEMENT

I, express my sincere gratitude and indebtedness to Dr. Rajesh Dhankhar, Department of Environmental Sciences, M.D.U. Rohtak, Haryana-India for her guidance and assistance in this research work.

REFERENCES

- Ballantyne, D.J. (1973). Sulphite inhibition of ATP formation in plant mitochondria. *Phytochem*., **12:** 1207-1209.
- Ballantyne, D.J. and Black, M.W. (1980). Sulphite stimulation of bean mitochondrial adenosine triphosphatase. *Phytochem*. **19:** 2021.
- Baruah AK, Sharma RN, Borah GC. Impact of sugar mill anddistillery effluent on water quality of river Galabil, Assam. Indian J. Environ. Health.2018; 35: 288–293.
- Benavides MP, Gallego SM, Tomaro ML. Cadmium toxicityin plants. Braz. J. Plant Physiol. 2015; 17: 49-55.
- Blackman, W.J. and Moreland, D.E. (1971). Adenosine triphosphatase activity associated with Mung bean mitochondria. *Plant Physiol*. **47:** 532-536.
- Cabral JR, Freitas PSL, Bertonha A, Muniz AS .Effects of wastewater from a cassava industry on soil chemistryand crop yield of lopsided oats (Avena strigosa Schreb.). Brazilian Archives of Biology and Technology .2020; 53:19-26.
- Carley, H.E and Watson R.D(1968) Effect of various aqoues plan extracts upon seed germination. *Bot. Gazet*,129(1):57-62
- CPCB (1995) . Pollution control acts, rules and notifications issued thereunder, *Cont. Poll.Cont.Board*, New Delhi.
- Chhonkar, P.K., Dutta, S.P., Joshi, H.C. and Pathak, H. (2000a). Impact of industrial effluents on soil health and agriculture – Indian experience: Part 1 – Distillery and Paper Mill Effluents.

Journal Sci. and Industrial Res., **59(5):** 350-361.

- Chhonkar, P.K., Dutta, S.P., Joshi, H.C. and Pathak, H. (2000b). Impact of industrial effluents on soil health and agriculture – Indian experience: Part 11 – Tannery and Textile Industrial Effluents. *Journal Sci. and Industrial Res.,* **59(6)**: 446-454.
- Demirevska-Kepova K, Simova-stoilova L, Stoyanova Z, Feller U. Cadmium stress in barley: Growth, leaf pigment, and protein composition and detoxification of reactive oxygen species. J. Plant Nutr. 2016; 29: 451-468.
- Dubey, R.S. (1987). Effect of NaCl salinity on the enzyme activity and biochemical constituent in germinating salt tolerant rice seeds. *Crop Physiol. Abst.*, **13:** 20.
- Ebukanson, G.J. (1987). Retardation of chloroplast ATPase activityin maize seedling by drought stress. *J. Plant Physiol*., **129:** 187-189.
- Hayat, S, Ahmad I, Azam ZM, Ahmad A, Inam Samiullah A. Effect of longterm application of oil refinery wastewater on soil health with special reference to microbiological characteristics. Bioresource Technol.2021; 84:159-163.
- Jamali MK, Kazi TG, Arain MB, Afridi HI, Jalbani NMemon AR. Heavy metal contents of vegetables grownin soil irrigated with mixtures of wastewater and sewagesludge in Pakistan using ultrasonic-assisted pseudo- digestion. J. Agron. Crop. Sci.2017; 193: 218
- Kashem MA, Singh BR. Heavy metal contamination of soil and vegetation in the vicinity of industries of Bangladesh. Water Air Soil Pollut.2019; 115: 347-361.
- Kalra N. and Joshi H.C., Chaudhary A., Choudhary R. and Sharma S.K. (1997). Impact of flyash incorporation in soil on germination of crops. *Bioresource Technology.* **61:** 39-41.
- Khan H.J. (2000). Clean-up act. Science Reporter. **37(4):** 42-43.
- Konwar D, Jha DK. Response of Rice (Oryza sativa L.) toContamination of Soil with Refinery Effluents under Natural Conditions. Biological and Environmental Sciences. 2019; 5(1):14-22.
- Masarovicova E, Cicak A, Stefanick I. Plant responses to air pollution and heavy metal stresses. In: Pessarakli M, ed. Handbook of Plant and Crop Stress. 2nd ed. NewYork.Marcel Dekker. 2019:569–598
- Mengel K., Kirkby EA, Kosegarten H, Appel T. Principles of plant nutrition, 5th Ed., Springer, Heidelberg .2020.
- Prashanthi, V. and Rao, K.J. (1999). Impact of land disposal of industrial effluents on properties of soils of Noor Mohammed lake bed. *J. Environmental Bio.,* **20(3):** 271-274.
- Sangwan, S. and Dhankhar, R. (2010). Biomass of Aspergillus sydowi act as a bioadsorbent for removal of heavy metals from refinery effluent. *Journal of Interdisciplinary and Multidisciplinary Research (JIMR)*: **5(7): 33-42**
- Sangwan, S. and Dhankhar, R. (2016). Removal of Cr, Ni and Pb from Refinery effluent by Using Wheat Straw bio-adsorbant. *International Research Journalof Natural and Applied Sciences*: **3(12): 197-204**
- Sangwan, S., Sushma and Dhankhar, R. (2016). Analysis of Panipat Oil Refinery Effluent- A Case study. *Journal of Interdisciplinary and Multidisciplinary Research (JIMR)*: **5(7): 33-42**
- Sharma, S.K., Karlra, N. and Singh G.R. (2001). Impact of coal-burnt ash on environment and crop productivity. *J. Sci. Indi. Res.***60:** 580-585.
- Sharma, S.K., Kalra, N. and Singh G.R. (2002). Soil physical and chemical properties as influenced by flyash addition in soil and yield of wheat. *J. Sci. Ind. Res.,* **61:** 617-620.

- Ranganathan, R., Sheela, R., Venkatesan, A., and Ravindran, K.C. (1999). Sea water effect on growth and photosynthetic parameters of *Avicennia oficinalis L.*, *Geobios,* **26(4):** 179-182.
- Rao, A.P. and Rao, P.V.V.P. (2002). Pollution potential of sago industry: A case study. *J. Ecotoxicology Environmental Monitoring,* **12(1):** 53-56.
- Fiske, C.H. and Subbaraw (1925). The colorimetric determination of phosphorous. *J. Bio.Chem.*, 66: 375-400.
- Mittal, R. and Dubey, R.S. (1992). Mitochondrial acid phosphatase and *Adenosine triphosphatase* activities in germinating rice seeds following NaCl salinity stress. *Ind. J. Plant Physiol.*, 35(2): 174-181.
- Palmgren, M.G. (19990). An H^+ -ATPase assay; Proton pump and ATPase assay**:** Proton pump and ATPase activity determined simultaneously in the sample, *Plant Physiol*., **94:** 882-886.
- Sen, S. and Mukherji, S. (2004). Alterations in activities of acid phosphatase, alkaline phosphate, ATPase and ATP convent in response to seasonally varying. Pi status in Okra (*Abelmoschus esculentus*). *J. Env.. Bio.,*
- Sokal, R.R. and Rohlf, E.F. (1995). Biometry: The principles and practice of statistics in biological research, 2nd ed. W.H. Freeman, New York.