EFFECT OF INDUSTRIAL EFFLUENTS ON LEAF SENESCENCE OF CROP PLANTS

Dr. Sagarika Nayak, KISS, KIIT, Bhubaneswar

Abstract

Senescence is predetermined and is genetically controlled natural phenomenon in plants. This phenomenon leads either the whole plant or its various organs such as leaves, flowers, fruits at the microscopic level shrivel and chlorophyll, protein and nucleic acids at the microscopic level are reduced at a particular time of their life cycle. Ageing and senescence are the general terms used in scientific purposes. Senescence is defined as the deteriorate process that are natural causes of death. Ageing by contrast, refers to the processes of occurring maturity with the passage of time. In short ageing and senescence are fundamental intrinsic properties of living organisms. Since length of period of retention of green colour is related to yield in crop plants. Senescence and its study assumes a greater importance in agriculture. Several workers have studied the influence of various chemicals on leaf senescence in crop plants like rice, wheat, oat, Maize, Ragi etc. A variety of chemicals and industrial pollutants have effect on leaf senescence of crop plants.

Key words : Senescence, Ageing, Chlorophyll, Deteriorate

1. INTRODUCTION

1.1 senescence

Senescence is defined as those reproducible time related alternations in structure and function in an organism which result in the decreasing capacity of that organism to survive thus result ultimately to its death. The increasing production of accumulation of a senescence factor which has been as much genetically programmed as the process of development, growth and maturation and the gradual diminution in production of a "JUVESCENT" substances and its ultimate failure altogether, also genetically predetermined, which can be suggested as being responsible for programmed senescence. On the other hand ageing is a process, not a discrete event. This implies that it is a sum of many events, probably at the molecular level and of at least several different kinds. These changes slowly decrease the fitness of the organism. Although many gradual changes occur with time, the changes of importance to the subject of biological ageing are those which decrease the capacity of the organism to survive the challenges of the environment, internal or external. Any broad definition of ageing must include the elements of time dependency of reproducibly observable changes in structure and function in an organism. Consideration of the process of ageing must there fore cover all three major phases of existence of any organism i.e.

a. the initial period of embryonic development

b. the middle life stage of growth and maturation

c. the period of senescence.

So senescence as a more restricted part of the total ageing process. It can be considered as those changes in structure and function which are deleterious and degenerative, so that, in a time related sense such changes would result ultimately the failure of the individual to survive and ultimately its death.

1.2 Leaf senescence

It is the characteristic feature of the plant organization that some parts enter senescence and die long before the orders. Growth of a main axis with a relatively long life which bear its different determinate organs like branches, leaves, flowers and fruits which have limited power of growths and limited span of life. Leaf as a determinate organ needs special mention here, not because as it is having the ability to reserve the process of senescence unlike other determinate organs which exhibit permanent and irreversible changes during senescence. So leaf senescence is a complicated process controlled by a number of endogenous and exogenous factors and it is classified as:

a. Attached leaf senescence

b. excised leaf senescence

1. 3 excised leaf senescence

The changes which take place during the senescence of attached leaves are multiple in nature and these changes are accelerated when the leaves are detached from the plant and allowed to senescence artificially. Most of the senescence studies have been done using excised leaves (Bajwa et al.2013, Chen et al. 2012, Byeon, 2015, Greer et al.2011). Since senescence is an inevitable event in the life cycle of a living organism, there developed an urge to know the detailed sequence of changes taking place from the onset of senescence till complete senescence in the organ/plant. A relative influence of a variety of chemicals and environmental factors on senescence to be find out through scientific study. The other investigations are to be done to find out if there is any environmental factors or a chemical of a particular strength to prevent the senescence process and for lengthening the life span of plants as well as for increasing production.So the study of senescence thus assumed greater importance in agriculture.

1.4 Effect of an industrial effluents in excised Ragi leaf senescence

Ragi (Elusine coracana Gaertn cv 202) is a staple food crop with proteineous substances which is used by maximum people of south Odisha and much useful for diabetic persons both as rabi and Khariff crop to meat the protein need of the south Odisha's poor cultivators and especially the poor Adivasi people of south Odisha. There fore there is a need to study the effect of different chemicals on senescence in plants particularly in cereal crop such as Ragi.

Advancement of a country is known by the degree of industrialization . Industrialisation is taken as one parameter to mark the advancement of a nation. To avoid pollution, industrial effluents are to be properly recycled .The effluent of the industries are discharged into the river Rusikulya is also left into Ragi fields either directly or indirectly as the Ragi and other crops are irrigated by the river water. Very few studies have done regarding the composition of effluent and its effect on the senescence of Ragi plant. Therefore the present study is concerned with the influence of the industrial effluent on changes in macro molecules and activity of different enzymes associated with senescence in excised leaves of Ragi commonly grown in this area by the farmers.

2.0 MATERIALS AND METHODS

2.1 Eleusine coracana Gaertn cv.202

Eleusine coracana Gaertn cv.202 was used as experimental material in the present investigation Pure line seeds of the Ragi under study were obtained from the farmers of the industrial area. Seeds of uniform size and colour were selected and were surface sterilized with 0.03% formalin solution for one hour and then washed thoroughly with tap water and then with distilled water. The seeds were then put in the sterilized petridishes lined with moist blotting papper at laboratory temperature. Care was taken to maintain uniform moisture in petridishes .Better sprouted 5 days old seedlings were transplanted to earth ware pots containing a well puddle mixture of Ragi field soil and cowdung manure mixed in a proportion of 8:1 parts by volume. There were about 20 seedlings in each pot (25x25x10 cm in size).

Healthy green and well expanded fourth leaf was collected from 30 days old Ragi plant and were washed with distilled water. These leaves were made into different sets to be floated on 100 ml. distilled water of aqueous solutions of the effluent of different concentrations and were kept incubated for the required period of experimentation. The leaves from the petridishes during incubation have been sampled initially and at intervals for estimation like chliorophyll, protein, nucleic acids (DNA and RNA), catalase and peroxidase activity.

2.2 Continuous irradiation with white light,

It was provided by 2 fluorescent tubes (Phillips TL40 Watt/volt) giving an incident intensity of 2000 lux at the plant leaf level in a light chamber specially prepared for the purpose.Transfer of petridishes to dark chamber and natural condition with leaf material were done simultaneously to study the effect of effluents in darkness and natural photoperiod. During incubation of leaves, samples were taken initially and at intervals for biochemical and enzyme analysis.A light, natural photo period and dark control was kept separately as controls for comparision.

2.3 Study of effluent effect

The effluents from industries which are discharged into the nearby Ragi fields or into the river water which is used for irrigation purpose in the nearby Ragi fields was collected for present study. The chemical characteristics of effluent (gm/l) discharged from the industry near Ganjam are as follows:

Sodium : 4000 ± 1000, Potassium: 600 ± 180, Calcium: 65 ± 07, Magnesium:40 ± 04, Mercury: 0.2 ± 0.2, Chloride:16000 ± 3000, Phosphorus: 47 ± 0.5.

2.4 Selection of effective Concentration of effluent

Yellowing is the distinct indication of senescence. Yellowing is caused by chlorophyll loss. The effectiveness of any exogenous agent by its capacity to delay the yellowing process. So an effective concentration of the agent is the first requirement in the investigation to be carried on. There is an optimum value below or above which the senescence retarding capacity may decrease or even show opposite effects. Keeping in view of this fact, a preliminary screening has been performed to select the optimum concentration of the effluent. The selected optimum concentration was used for the further experiments. Leaf samples were collected, worked and randomized. The samples (Ca 50 mg. fresh wt.) were floated in petridishes containing 100 ml aqueous solution of the effluent The concentrations (v/v)of the effluent taken for the purpose were 2.5,5.0,10.0,20.0 and 25.0.The experimentation were carried in natural photo period.

2.5 Collection of data

Data collected are the mean of 5 replicates for different parameters in different experiments and were subjected to statistical analysis for standard error .The detailed methodology of estimating the macromolecular components and assaying the enzymes is given here under.

2.6 Extraction and estimation of macromolecular contents and assay of enzyme activity

Leaves were harvested from the treatments at 48 hr intervals till the eighth day. Harvested leaves were used for the study of changes in the macromolecular contents and enzymatic activities.

Each time immediately after harvest, the leaves were washed with distilled water and leaf samples weighing (ca 50 mg fresh weight) were used.

2.6.1 Extraction and estimation of chlorophyll

Chlorophyll was extracted and estimated by following the method of Osborne (1962).Leaf material were grinded with 80% (v/v) once with the ethanol and after centrifugation at 5000xg. The remaining chlorophyll was again extracted from the pellet treating 80% (v/v) boiling ethanol two times. Chlorophyll content of the extracts was determined by measuring the absorbance of the extracts at 665 nm in a photoelectric digital spectrophotometer.

2.6.2 Extraction and estimation of protein

Protein was estimated by the method of Lowry et al. (1951).Bovin serum albumin was used as the reference standard. The milk white precipitate after chlorophyll extraction were suspended in 5% (w/v) TCA at 0°C for 15 minute and centrifuged at 10,000xg for 20 min. The process was repeated twice. Then 2ml NaCl was added to each residue present in the tubes .The tubes were left as such at room temperature for 30 min and then kept in boiling water bath for 15 minutes.

2.6.3 Extraction and estimation of nucleic acid

The estimation was done following the method of Schneider (1957).The milk white precipitate after chlorophyll extraction was suspended in 5% (w/v)TCA at 0°C for 15 minute and centrifuged at 5000xg for 20 min at 4°C and the process was repeated twice.This was followed by one extraction with absolute ethanol, two extractions with ethanol/ether and chloroform.The residues were then suspended for 20 minutes in μ ml 10 % (w/v) hot TCA at 90°C for nucleic acid extraction .The DNA and RNA contents of the TCA extracts after cooling were determined by the diphenylamine and orcine respectively.

2.6.4 Extraction and assay of catalase (ECI. 11.1.6)

The leaf material was ground with 8ml. 0.1m phosphate buffer pH 7.0 with a pre chilled mortar and pestle and the homogenate was centrifused at 15000xg for 30 mins at 4°C and the aliquot was used as the source of catalase.Catalase activity was arranged by modified method of Kar and Mishra (1976).One ml enzyme extract was added to 2 ml 0.005M H_2O_2 and 1ml 0.1M phosphate buffer of pH 7.0.The reaction was stopped by adding 10ml,1.0M sulphuric acid after 2 minutes incubation at 20°C. The residual H_2O_2 was titrated with 0.002M $KMnO_4$ v/l.A faint purple colour persisted for at least 15 second. A blank was prepared by adding the enzyme extract to an acidified solution of the reaction mixture of zero time catalase activity which was expressed as one mole H_2O_2 utilized (g/wt/m) i.e. one unit of catalase activity is defined as that amount of enzyme which breaks 1 mole of H_2O_2 per minute under the assay conditions described above.

2.6.5 Extraction and assay of peroxidase (EC1.11.1.7)

Extraction procedure of the enzyme was similar as that of catalase. Following the modified method of Kar and Mishra (1976) the activity of the enzyme was assayed. The assay mixture for peroxidase contained 2ml 0.01M pyrogallol, 1ml. of 0.005M H_2O_2 and 1 ml. dilute enzyme extract. After incubation of 25°C for 5 min the reaction was stopped by adding 1 ml, 2.0 M H_2SO_4 and the amount of pyrogallin formed was estimated by measuring the absorbance at 420nm. Peroxidase activity was expressed in absorbance units.

3.0 Experimental Results

An experiment was designed to study the effect of different concentration of the effluent v/v. Effluents discharged from chloroalkali factory at Ganjam was taken to study the effect of effluents on changes in chlorophyll content of 30 days old detached Ragi leaves under three environmental conditions namely light, natural and dark condition .

3.1 Changes in chlorophyll content of Ragi leaves in detached condition

The effluent brought about chlorophyll loss following an increase in the concentration of the effluent in a linear fashion. Further, there was a marked decline in the chlorophyll content following the increase in the incubation period as compared to the control irrespective of the concentration of the effluent. A significant chlorophyll loss was noticed in the leaves treated with effluent higher then 5.0 (v/v) strength. Loss of chlorophyll observed in all concentrations of effluent tried from 2.5(v/v) to 25.0(v/v).The effluent at a strength of 5.0(v/v) proved to be effective one as concentration below or above this value caused significant chlorophyll loss. Thus 5.0(v/v) of the effluent proved to be the optimum concentration to be taken up for further investigation.(Table 1).

3.2 Change in chlorophyll content

With regard to the changes in chlorophyll content, it was seen that the chlorophyll content in treated leaves remained at lower level than in the controlled ones throughout the period of study under all the three conditions tried. The effectiveness of the order light<natural <Dark (Table 2).

3.3 Changes in protein content

The protein in control leaves remained higher then the leaves treated with effluent. It was seen in all the three conditions of treatment and incubation with regard to the effectiveness of the conditions influencing the protein loss in light it was found to be less and effectiveness in dark was found to be most. An in between loss was observed in natural condition. But in all the three condition there was a gradual decline in the protein content with the increase of incubation period after all the conditions tried (Table 3).

3.4 Changes in DNA content

Changes in DNA content in Ragi leaves treated with the effectors decline from the start of the experiment in all three conditions. The relative effectiveness of the conditions in altering the content of macromolecular substance is in the order of light < Natural < Dark. In all conditions controlled leaves maintained higher DNA content than the leaves treated with the effluent (Table 4).

3.5 Changes in RNA content

RNA content in senescing leaves showed a sharp fall following an increase in the incubation period irrespective of the experimental condition. Control treated leaves showed higher RNA content level than the leaves treated with effluent .The relative effect of the experimental conditions causing changes in RNA content being Light>Natural>Dark (Table 5).

3.6 Changes in catalase activity

The catalase activity was found to be declining in all the treated and control leave throughout the period of experimentation. Catalase activity in control leaves was higher then the treated ones irrespective of the experimental conditions. The effectiveness of the conditions in decreasing the activity of the enzyme being light <Natural <dark (Table 6).

3.7 Changes in peroxidase activity

An altogether different picture was noticed with respect to peroxidase activity in control leaves maintained along side the leaves treated with the effluent. Peroxidase activity in the control leaves remained far below the value recorded in treated leaves at each time of the conditions in maintaining the peroxidase activity was in the order of Light >Natural >Dark (Table 7)

4. Discussion

4.1 Role of Light

Senescence process in the excised leaves causes the grdual loss of green colour and turns it into yellow due to disintegration of cellular structure (Guo. 2012, Brusslan et al. 2012, Peleg, 2011,Scarpeci et al. 2013).The loss of chlorophyll was found to be more in dark than in continuous light and it is intermediate in natural condition. In view of light dependent retardation of chlorophyll loss, a phytochrome mediated control of senescence was suggested It has been observed that far red form of phytochrome controls a large number of enzymes in the cell. light acts by synthesizing certain growth regulators like cytokinin and darkness in synthesizing a senescence factor. light is the efficient senescence retarding agent in Ragi. As regards the intensity and duration of light it was found that continuous light with intensity Ca 200 iux inhibit senescence efficiently compared with natural light and continuous dark. In the present investigation it is reported that light acts as a powerful senescence retardant.

4.2 Role of the industrial effluent

Industrial effluent is a heterogeneous mixture with many ions and radicals with varying concentration . Hence an attempt is made to correlate the findings with the salt stress induced changes in different plants with the earlier available reports to analyse the possible synergetic effect of different ions on the different metabolic processes in plants or leaves.Chloroplast protein is responsible for the synthesis of chlorophyll, the loss of it (chloroplast protein) might be the reason for the corresponding loss in the chlorophyll pigment in the leaves. Further it was reported that pigment-protein lipid complex breaks down resulting in the destruction of chlorophyll pigment.Under salt stress conditions, pertaining to weak stability of the bond of chlorophyll in the protein lipid complex was earlier observed by (Lopes et al 2012, Metallana et al. 2013, Merewitz et al.2011, Nie et al. 2012). Further investigation done by many scientists

suggested that the ions present in the effluent accelerated the inhibition of chlorophyll synthesis in light, natural and dark condition as compared to respective controls. A positive correlation was drawn between the chlorophyll loss and incubation period as well as the concentration of the effluent. Since the leaves are incubated in excised condition the recovery of leaves from the harmful effect of the effluent is not possible as a result a rapid decline in chlorophyll content was noticed during the process of incubation.

4.3 Interaction of Effluent in light, natural and dark conditions

As discussed in the chapter 4.1 light plays an important role in leaf senescence with regard to industrial effluent and light interaction a rapid degradation of chlorophyll was marked under the dark conditions than in natural and light condition .The degradation was significant in effluent treated leaves than in control. As pointed out in chapter 4.3, the different ionic concentration present in the effluents might have accelerated the degradation of chlorophyll. (Sakurba et al.2013, Scarpeci et al. 2013, Watanabe et al. 2013, Suzuki et al.2012,)

4.4 Changes in Macromolecular contents

During the process of senescence it was reported that the plant as a whole show a drastic change in the cellular and sub cellular constituents such as chlorophyll, protein and nucleic acids (RNA & DNA) etc.However the rate of change varies from plant to plant or species to species or mode or type of application of the chemicals and other stresses(Messmer et al.2011,Wu et al. 2012 Rauf et al. 2013, Rajniak et al.2015).Several workers also observed the chlorophyll breakdown of protein and concomitant amino acid accumulation. Like chlorophyll and proteins, nucleic acids both DNA and RNA contents also declined in senescence leaves. The DNA and RNA level during senescence is well documented in the present investigation. It was observed that RNA contents remained comparatively higher and stable than DNA contents (Lopes et al. 2012, Matallana et al. 2013, Merewitz et al. 2011)

As the effluent is a heterogeneous mixture of different ions, salt and radicals the effect of effluent on senescence of leaves can be discussed basing on the reports of previous observation by different workers. As chloroplast protein is responsible for synthesis of chlorophyll, the loss of it might be the reason for a corresponding loss in the chlorophyll pigments inside the senescing leaf tissues.It may be further said that the effluents might have induced alternation in protein metabolism leading to the loss of chloroplast protein. As reported by many scientists the salt stress conditions of effluent might have weakened the bonding of chlorophyll in protein – lipid complex leading to decrease in chlorophyll content.(Has et al.2012, Hickman et al.2013, Hunkova et al.2011, Lee et al. 2012, Liu, 2012). ln the present investigation a significant alteration in protein and nucleic acid show that the ionic concentration of different salts in the effluents are responsible for cellular degradation leading to earlier senescence than the control ones.(Vogelmann et al.2012,Komandare and rape, 2012, Lee et al. 2011, Liang, 2015, Liu, 2015,)

4.5 Changes in enzyme activity

Two peroxisomal enzymes like catalase and peroxidase are responsible for the removal of H2O2 from the plant tissue. As exogenous application of H2O2 enhances the process of senescence, it is believed that the two enzyme are very important in regulating the peroxidase level during the

process of senescence.The information available indicates the decrease in the activity of catalase in senesceing tissues (Vadez et al. 2011, Nelson et al. 2011, Nie et al. 2012, Suzuki & Makhino, 2013).In the present investigation a decrease in catalase activity was observed under the influence of the effluent under all three conditions of incubation (Table-6).

Peroxidase activity showed an increased trend in all the conditions of incubation during the senescence process. A photo induction raised peroxidase activity also reported by many scientists. In this piece of work a similar observation was recorded. Peroxidase is considered as reliable senescence induced activity of physiological process. But the functional role of the enzyme is not yet clearly known. An intensive study is necessary before ascertaining the role of enzyme in the physiological senescence. There are reports that molecules of catalase extracted from leaf tissue develop peroxidase activity.The type of conversion of tetrameric catalase molecules to monomeric peroxidase molecules was considered as one of the possible reason for the increase in peroxidase activity in senescing plant tissues. A close negative correlation between catalase and peroxidase enzyme in detached leaves of Ragi is also suggested by many scientists. Decreasing activity of catalase observed with the treatment of effluent was in agreement with salinity induced findings and senescence induced findings of different scientists (Selvaraj et al.2010, Selvaraj et al.2011,Selvarathi, 2010, Tohge et al. 2012).

In the present investigation the effluent effect is inhibiting the chlorophyll synthesis significantly related with catalase and peroxidase .The loss of chlorophyll being positively related with catalase activity and negatively related with peroxidase activity. It is due to the induction by the effluent, an ion deficit condition inside the senescing excised leaves.The presence of catalase in the living cell ensures a low peroxidase concentration and as such , its decrease as observed in the present study suggests probably increase in peroxidase content in the living plant .Hence decrease in catalase activity and simultaneous increase in peroxidase activity observed in the present investigation. Many scientists have suggested that catalase attribute a scavengers role to eradicate H2O2 .(Zhang et al.2011, Xu et al.2011, Zhang and Gan,2012)

A greater analytical study should be under taken with a variety of plants using different chemicals which are used in agriculture and which effects the agriculture like industrial effluent and other physiological studies were required to understand this complex phenomenon of senescence.

Table-1

Effect of different concentration of effluent on changes in total chlorophyll content of 30 days old detached Ragi leaves incubated in natural condition.

Each data is the mean of 5 replicates. Initial value : 0.41 absorbance units at 665 nm

Treatment	Concentration of chemical	Incubation period (days)			
		2	4	6	8
Control	Control	0.16	0.21	0.33	0.28
Effluent	2.5v/v	0.26	0.23	0.18	0.12
	5.0 v/v	0.20	0.18	0.12	0.06
	10.0 v/v	0.15	0.11	0.07	0.03
	20.0 v/v	0.10	0.05	0.03	0.01
	25.0 v/v	0.05	0.03	0.02	-

Table-2

Effect of different concentration of effluent on changes in total chlorophyll content of 30 days old detached Ragi leaves incubated in light, natural and dark condition.

Each data is the mean of 5 replicates ± S.E. Initial value :0.41 absorbance units at 665 nm

Condition	Treatment	Incubation period (Days)			
		2	4	6	8
Light	**Control**	0.13	0.28	0.23	0.18
	Effluent	0.17	0.13	0.08	0.02
Natural	**Control**	0.24	0.20	0.14	0.06
	Effluent	0.14	0.11	0.06	-
Dark	**Control**	0.19	0.08	0.06	0.04
	Effluent	0.12	0.04	0.02	-

Table-3

Effect of different concentration of effluent on changes in total protein content of 30 days old detached Ragi leaves incubated in light,natural and dark condition.

Each data is the mean of 5 replicates ± S.E

Initial value :18.56 ± 0.12

Condition	**Treatment**	**Protein content mg/g fresh weight**			
		Incubation period (Days)			
		2	4	6	8
Light	Control	14.5	9.10	6.23	3.18
		± 0.10	±0.11	±0.09	±0.05
	Effluent	8.91	5.12	3.03	1.58
		± 0.15	± 0.11	± 0.05	± 0.09
Natural	Control	13.8	10.12	8.28	5.98
		± 0.2	± 0.17	± 0.08	± 0.07
	Effluent	6.15	3.82	9.92	-
		± 0.12	± 0.10	± 0.07	-
Dark	Control	13.29	7.89	3.91	1.82
		±0.16	± 0.11	±0.08	± 0.11
	Effluent	3.58	2.29	1.23	-
		± 0.20	±0.11	± 0.07	-

Table-4

Effect of different concentration of effluent on changes in DNA content of 30 days old detached Ragi leaves incubated in light, natural and dark condition.

Each data is the mean of 5 replicates ± S.E

Initial value :0.22 ± 0.02

Condition	Treatment	DNA content mg/g fresh weight			
		Incubation period (Days)			
		2	4	6	8
Light	Control	0.20	0.16	0.11	0.05
		± 0.01	±0.02	±0.01	±0.01
	Effluent	0.11	0.06	0.03	-
		± 0.01	± 0.02	± 0.01	-
Natural	Control	0.17	0.12	0.05	0.02
		± 0.02	± 0.01	± 0.01	± 0.01
	Effluent	0.09	0.05	0.03	-
		± 0.01	± 0.01	± 0.01	-
Dark	Control	0.13	0.10	0.06	-
		±0.01	± 0.01	±0.01	-
	Effluent	0.16	0.03	0.02	0.01
		± 0.20	±0.11	± 0.07	±0.01

Table-5

Effect of different concentration of effluent on changes in RNA content of 30 days old detached Ragi leaves incubated in light,natural and dark condition.

Each data is the mean of 5 replicates ± S.E

Initial value :0.22 ± 0.02

Condition	Treatment	RNA content mg/g fresh weight			
		Incubation period (Days)			
		2	4	6	8
Light	**Control**	1.11	0.12	0.51	0.33
		± 0.02	±0.02	±0.02	±0.01
	Effluent	0.88	0.80	0.32	0.19
		± 0.01	± 0.01	± 0.01	± 0.01
Natural	**Control**	21.18	0.90	0.42	0.13
		± 0.02	± 0.01	± 0.01	± 0.01
	Effluent	0.74	0.38	0.19	-
		± 0.01	± 0.01	± 0.01	-
Dark	**Control**	1.28	0.79	0.33	-
		±0.01	± 0.01	±0.01	-
	Effluent	0.38	0.22	0.07	-
		± 0.02	±0.01	± 0.01	-

Table-6

Effect of different concentration of effluent on catalase activity of 30 days old detached Ragi leaves incubated in light, natural and dark condition.

Each data is the mean of 5 replicates ± S.E

Initial value :3.20 ± 0.03

Condition	Treatment	Catalase activity 1m mole of H2O2 Utilised			
		Incubation period (days)			
		2	4	6	8
Light	Control	2.22 ±0.03	1.39 ±0.04	0.79 ±0.03	0.80 0.03
	Effluent	0.96 ±0.03	0.74 ±0.03	0.38 ±0.03	0.09 ±0.01
Natural	Control	2.28 ±0.05	0.97 ±0.03	0.67 ±0.02	0.54 ±0.01
	Effluent	0.74 ±0.01	0.38 ±0.01	0.19 ±0.01	-
Dark	Control	1.28 ±0.01	0.79 ±0.01	0.33 ±0.01	-
	Effluent	0.38 ±0.02	0.22 ±0.01	0.07 ±0.01	-

Table-7

Effect of different concentration of effluent on peroxidase activity of 30 days old detached Ragi leaves incubated in light, natural and dark condition.

Each data is the mean of 5 replicates ± S.E

Initial value :3.20 ± 0.03

Condition	Treatment	RNA content mg/g fresh weight			
		Incubation period (Days)			
		2	4	6	8
Light	Control	2.22	1.39	0.79	0.80
		± 0.03	±0.04	±0.03	±0.03
	Effluent	0.96	0.74	0.38	0.09
		± 0.03	± 0.03	± 0.03	± 0.01
Natural	Control	2.28	0.97	0.67	0.54
		± 0.05	± 0.03	± 0.02	± 0.01
	Effluent	0.82	0.64	0.29	-
		± 0.03	± 0.06	± 0.01	-
Dark	Control	2.92	1.48	0.92	0.30
		±0.03	± 0.03	±0.03	±0.03
	Effluent	0.84	0.43	0.32	-
		± 0.02	±0.01	± 0.01	-

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