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Research Article

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Effect of rice mill effluent on growth and biochemical parameters of *Lycopersicon esculentum* in pot culture

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ABSTRACT

The present study showed that rice mill effluent had an adverse effect on growth and development of tomato plant at higher concentration, but in lower concentration there is no toxic effect on general welfare, rather it has some beneficial effect on the growth and development of the crops in certain concentration of effluent. The continued importance of tomato (Lycopersicon esculentum Mill.) as a vegetable and salad commodity is reflected by the large volume of research on virtually all aspects of the crop. In the present paper, the physicochemical characteristics of rice mill wastewater were measured prior to field experiment. The wastewater revealed an alkaline pH (7.51) with low concentration of DO (1.2 mgl^{-1}), nitrate (0.76 mg l^{-1}), phosphate (18.1 mg l^{-1}) and sulphate (37.66 mg l^{-1}) and moderate concentration of COD (513 mg l^{-1}), chloride (121.5 mg l^{-1}) and TDS (562.63 mg l^{-1}) and high concentration of total suspended solids (536.66 mg l^{-1}) and BOD (313.7 mg l^{-1}). In our present investigation, beneficial impact on general welfare of the tomato plant was gradually increased with increasing the concentration of the effluent up to 100 ppm.

Key words: *Vigour index, Protein, Carbohydrate, Amino acid*

INTRODUCTION

A significant impact of globalization on horticulture has been an increasing demand for quality improvement and the wider adoption of quality standards for fruit, vegetable and salad commodities[14].Tomato (*Lycopersicon esculentum* Mill.) is one of the important vegetable crops grown throughout the world and ranks next to potato in terms of the area cultivated but ranks first as a processing crop[8]. The total estimated annual global production is over 120 million metric tons (FAO, 2007). It is one of the most widely used food crops in world vegetable economy[4]. Tamato is particularly rich in pigments and secondary metabolites like lycopene (red pigment in tomato fruit) - a strong anti carcinogen, and Vitamin. Parboiling is a premilling process for paddy which originated in India. All the industries have a social responsibility to protect the environment for future generation. Rice mill industry is one of the largest industries of our country. This is an important agriculture based industry in India. These industries are also an important source of Indian economy. With increasing global population, the gap between the supply and demand for water is widening and is reaching such alarming levels that in some parts of the world it is posing a threat to human existence.

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Scientists around the globe are working on new ways of conserving water. Wastewater and its nutrient content can be used extensively for irrigation and other ecosystem services. Its reuse can deliver positive benefits to the farming community, society, and municipalities.

One of the ways to recycle water is to focus on reuse of urban and industries wastewater, for irrigation and other purposes. This could release clean water for use in other sectors that need fresh water and provide water to sectors that can utilize wastewater e.g., for irrigation and other ecosystem services. In developed countries where environmental standards are applied, much of the wastewater is treated prior to use for irrigation of fodder, fiber, and seed crops and, to a limited extent, for the irrigation of orchards, vineyards, and other crops.

Objectives

The objective is to provide a review of the characteristics of wastewater reuse for agriculture. Determination of rice mill effluent on growth and development of tomato plants & to determine a suitable concentration for best yield and fruit quality. To observe the variation in Photosynthetic pigment contents of tomato seedling grown under various concentrations of rice mill effluent. And also variation in biochemical contents of tomato seedling grown under various concentrations of rice mill effluent.

MATERIALS AND METHODS

Test species:

Tomato (*Lycopersicon esculentum*)

The tomato seeds were procured from the local seed supplier. The effect of rice mill effluents was studied on tomato pahuja no. 1 variety of tomato.

Rice mill effluent:

The rice mill effluents used in the present study were collected in pre cleaned containers from the Akrura, unit IV rice mill located near Pandakital, sonepur (Odisha), India.

Plant material & growth condition:-

Pot culture experiment:

Pots of same size were filled with equal amounts of sandy loam soil of medium fertility and 20 seeds of tomato were sown in each pot. The pots were irrigated with selected concentrations of the rice mill effluents. For each treatment, 100ml of each of these was applied to the respective pot at 5-day interval, throughout the study period. A control set, irrigated with distilled water was also maintained for comparison.

Physico-chemical Characteristics of the Rice Mill Effluent:

The physicochemical characteristics of rice mill effluent were discussed by following methods. In the present study pH has been measured soon after the collection of samples by a pH meter by electrometric method. Simultaneously the temperature is also measured by the systronic pH meter .Conductivity of the different samples has been measured using a conductivity meter by electrometric method. Winkler iodometric method was used for the determination of dissolved oxygen in water samples.The analysis of the sample was done on the basis of standard methods suggested by the American Public Health Association. Total Solid content was determined by Gravimetric method. In the present study chloride content was determined by Argentometric method. Total hardness of the samples was analyzed by titration with EDTA using Erichome black-T as an indicator.Total alkalinity and alkalinity due to carbonates, bicarbonates were estimated by titrating the sample with diluted H_2SO_4 using phenolphthalein as an indicator to pH 8.3 and methyl orange as an indicator to pH 4.2 and 5.4.The titrate value gives phenolphthalein alkalinity (PA) and the second one gives total alkalinity(TA). COD is determined by using reflux condenser, ferroin indicator and titrating the sample with ferrous ammonium sulphate. Nitrate and phosphate can be determined by spectrophotometric method.

Morphological Studies:

Method for Germination percentage:

The number of seeds germinated in each treatment was counted on 10th day after sowing and the germination percentage was calculated by using the following formula.

Germination % = No. of seeds germinated / Total No. of seeds sown x 100

Root and shoot length:

Five seedlings were taken from each treatment and their shoot and root lengths in cm were measured by using a measuring ruler and the values were recorded.

Seed vigour index:

SVI (Seed vigour index) = % of germination x Seedling length (cm)

Biochemical study:

Chlorophyll was estimated by Arnon, 1949, Carotenoids by Kirk and Allen,1965. Estimation of total protein done by Lowry et al., 1951. Estimation of total free amino acids carried out by Moore and Stein, 1948 and Carbohydrate by Yemm and Wills, 1954.

RESULTS

Each experiment was repeated three times. The data are expressed as mean (n=3). The data were analysed by ANOVA using MS Excel software.

The effect of rice mill effluent on the germination of seeds of tomato plant after 10 days of the beginning process of germination was observed & the data was recorded (Fig 1). The germination rate in Control treated plant was found to be 90% whereas seed treated with 50 mll^{-1}, 100 mll^{-1} & 200 mll^{-1} of rice mill effluent was found to be 92%, 88% & 82% (Fig 1). The tomato seeds treated with 50 mll^{-1} shows maximum germination.

The present study reveals that the seed vigour index of tomato plant was maximum at 100 ppm concentration i.e. 303.6 on 15th day. On 30th and 45th day the SVI was maximum at 50 ppm concentration i.e. 1054.32 and 1882.32 and then decreases as the concentration of the effluent increases .

The root length showed an increasing trend over control up to 100 ppm concentration of effluent applied soil and thereafter, it was decreased. The maximum root length was found at 100 ppm concentration i.e. 3.93 cm and 6.16 cm on 15th day and 30th day respectively but on 45th day the control shows the maximum root length i.e. 8.6 cm. The minimum root length was found at 200 ppm i.e. 3 cm, 5.4 cm, 8.6 cm on 15th, 30th and 45th day respectively.

The shoot length showed an increasing trend over control up to 100 ppm concentration of effluent applied soil and thereafter, it was decreased. The maximum shoot length was found at 100 ppm concentration i.e. 3.5 cm, 11.56 cm and 20.7 cm on 15th, 30th and 45th day respectively The minimum shoot length was found at 200 ppm i.e. 2.86 cm, 10.83 cm, 19.16 cm on 15th, 30th and 45th day respectively (Table 3, Fig 3). When the data was subjected to ANOVA, It shows significant differencewith concentration i.e. 10.29(Fc) > 4.75(Ft) as well as in increase in day 5131.83(Fc) > 5.14(Ft).

Biochemical analysis:

The biochemical contents increased up to 100 ppm concentration and then decreased in the higher concentrations.

Carbohydrate:

The highest carbohydrates content of root was found at 50 ppm concentration i.e. 2.796 mg/g fr. wt. on 15th day, at 100 ppm concentration i.e. 2.532 mg/g fr. wt. and 3.405 mg/g fr. wt. on 30th and 45th day respectively. The lowest carbohydrate content of root was found at control i.e. 2.297 mg/g fr. wt. On 15th day at 200 ppm i.e. 2.032 mg/g fr. wt. and 2.504 mg/g fr. wt. on 30th and 45th day respectively (Table 4, Fig 4). When the data was subjected to ANOVA, It shows no significant difference with concentration i.e. 1.84(Fc) < 4.75(Ft) but shows significant difference within increase in day 6.92(Fc) > 5.14(Ft).

The highest carbohydrates contents of shoot was found at 50 ppm concentration i.e. 2.904 mg/g fr. wt. on 15th day, at 100 ppm concentration i.e. 3.21 mg/g fr. wt. and 4.101 mg/g fr. wt. on 30th and 45th day respectively. The lowest carbohydrate content of shoot was found at control i.e. 2.452 mg/g fr. wt. on 15th day at 200 ppm i.e. 2.908 mg/g fr. wt. and 3.112 mg/g fr. wt. on 30th and 45th day respectively (Table 4, Fig 5). When the data was subjected to ANOVA, It shows no significant difference with concentration i.e. 0.85(Fc) < 4.75(Ft) and shows significant difference with increase in day 12.79(Fc) < 5.14(Ft).

Protein:

On 15th day the protein content of root is maximum at 50 ppm concentration i.e. 0.691 mg/g fr. wt. and minimum at 200 ppm i.e. 0.416 mg/g fr. wt. On 30th and 45th day the maximum protein content of root was found in control i.e. 1.669 mg/g fr. wt. and 1.971 mg/g fr. wt. respectively and minimum at 200 ppm i.e. 0.521 mg/g fr. wt. and 0.595 mg/g fr. wt. on 30th and 45th day respectively (Table 5, Fig 6). When the data was subjected to ANOVA, It shows significant difference with concentration i.e. 5.65(Fc) > 4.75(Ft) but does not shows significant difference with increase in day 4.94(Fc) > 5.14(Ft). The highest protein content of shoot was recorded at 100 ppm concentration i.e. 1.199 mg/g fr. wt., 1.392 mg/g fr. wt. And 1.804 mg/g fr. wt. on 15th, 30th and 45th day respectively. The lowest protein content of shoot was recorded at 200 ppm i.e. 1.064 mg/g fr. wt., 1.282 mg/g fr. wt. and 1.364 mg/g fr. wt. On 15th, 30th and 45th day respectively (Table 5, Fig 7). When the data was subjected to ANOVA, It shows no significant difference with concentration i.e. 3.75(Fc) < 4.75(Ft) but shows significant difference within increase in day 3.73(Fc) > 5.14(Ft).

Amino acid:

The highest amino acid content of root was found at 50 ppm concentration i.e. 0.073 mg/g fr. wt. on 15th day, at 100 ppm concentration i.e. 0.112 mg/g fr. wt. and 0.232 mg/g fr. wt. on 30th and 45th day respectively. The lowest amino acid content of root was found at 200 ppm i.e. 0.053 mg/g fr. wt., 0.091 mg/g fr. wt. and 0.203 mg/g fr. wt. as compared to control i.e. 0.062 mg/g fr. wt.,0.101 mg/g fr. wt. and 0.211 mg/g fr. wt. on 15th, 30th and 45th day respectively (Table 6, Fig 8). When the data was subjected to ANOVA, It shows significant difference with concentration i.e. 15.97(Fc) > 4.75(Ft) as well as in increase in day1318(Fc) > 5.14(Ft). The highest amino acid content of shoot was found at 50 ppm concentration i.e. 0.111 mg/g fr. wt. on 15th day, at 100 ppm concentration i.e. 0.223 mg/g fr.wt. and 0.427 mg/g fr. wt. on 30th and 45th day respectively. The lowest amino acid content of shoot was found at 200 ppm i.e. 0.091 mg/g fr. wt., 0.201 mg/g fr. wt. and 0.389 mg/g fr. wt. as compared to control i.e. 0.101 mg/g fr. wt. 0.211 mg/g fr. wt. and 0.408 mg/g fr. wt. on 15th, 30th and 45th day respectively (Table 6, Fig 9). When the data was subjected to ANOVA, It shows significant difference with concentration i.e. 7.24(Fc) > 4.75(Ft) as well as in increase in day 2174.38(Fc) > 5.14(Ft).

Pigment content analysis:

The chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content of tomato seedlings grown in different concentration of rice mill effluent are given in Table 7. The pigment contents showed a gradual increase over control up to 100 ppm concentration and decreased in the higher concentrations. The highest chlorophyll 'a' content was found at 100 ppm i.e. 0.612 mg/g fr. wt. and 0.756 mg/g fr. wt. on 15th and 30th day where as on 45th day the highest was found at 50 ppm i.e. 0.823 mg/g fr. wt. The lowest chlorophyll 'a' content was found at 200 ppm i.e. 0.256 mg/g fr. wt., 0.507 mg/g fr. wt., 0.712 mg/g fr. wt. as compared to control i.e. 0.52 mg/g fr. wt., 0.524 mg/g fr. wt. and 0.784 mg/g fr. wt. on 15th, 30th and 45th day respectively (Table 7, Fig 10). When the data was subjected to ANOVA, It shows no significant difference with concentration i.e. 3.3(Fc) < 4.75(Ft) but highly significant difference was found with increase in day i.e. 12.46(Fc) > 5.14(Ft). The highest chlorophyll 'b' content was found at 100 ppm i.e. 0.498 mg/g fr. wt. and 0.602 mg/g fr. wt. on 15th and 30th day where as on 45th day the highest was found at 50 ppm i.e. 0.746 mg/g fr. wt. The lowest chlorophyll 'b' content was found at 200 ppm i.e. 0.196 mg/g fr. wt. and 0.653 mg/g fr. wt. As compared to control i.e. 0.462 mg/g fr. wt. 0.71 mg/g fr. wt. on 15th and 45th day respectively whereas the lowest value was found in control on 30th day i.e. 0.327 mg/g fr. wt. (Table 7, Fig 11). When the data was subjected to ANOVA, It shows no significant difference with concentration i.e. 1.58(Fc) < 4.75(Ft) but significant difference was found with increase in day i.e. 8.94(Fc) > 5.14(Ft). The highest total chlorophyll content was found at 100 ppm i.e. 1.114 mg/g fr. wt. 1.247 mg/g fr. wt. on 15th and 30th day whereas on 45th day at 50 ppm the highest value was found i.e. 1.569 mg/g fr. wt. The lowest total chlorophyll content was found at 200 ppm i.e. 0.442 mg/g fr. wt., 0.993 mg/g fr. wt., 0.365 mg/g fr. wt. as compared to control i.e. 1.008 mg/g fr. wt., 0.857 mg/g fr. wt. and 1.494 mg/g fr. wt. on 15th, 30th and 45th day respectively (Table 7, Fig 12). When the data was subjected to ANOVA, It shows no significant difference with concentration i.e. 2.32(Fc) < 4.75(Ft) but highly significant difference was found with increase in day i.e. 10.93(Fc) > 5.14(Ft). The highest carotenoid content was found at 100 ppm i.e. 0.425 mg/g fr. wt. 0.407 mg/g fr. wt. on 15th and 30th day whereas on 45th day at 50 ppm the highest value was found i.e. 0.541 mg/g fr. wt. The lowest carotenoid content was found at 200 ppm i.e. 0.127 mg/g fr. wt. and 0.45mg/g fr. wt. as compared to control i.e.

0.383 mg/g fr. wt. and 0.493 mg/g fr. wt. on 15th and 45th day respectively whereas the lowest value was found in control on 30th day i.e. 0.276 mg/g fr. wt. (Table 7, Fig 13). When the data was subjected to ANOVA, It shows no significant difference with concentration i.e. 2.28(Fc) < 4.75(Ft) but highly significant difference was found with increase in day i.e. 5.34(Fc) > 5.14(Ft).

DISCUSSION

Rice industry is one of the largest industries in the world. It is one of the most important agro based industries in India and it is highly responsible for creating a significant impact on rural economy of the country as a whole. The rice mill industry generated a large volume of waste water during the manufacture of par boiled rice. Most of the rice mill industries and their ancillary units are responsible for polluting the water and the water bodies around them.

Physico-chemical properties of rice mill effluent:

The physico-chemical analyses of rice mill effluent showed that the effluent was alkaline in nature. It contained high amount of suspended solids and dissolved solids resulting in high Biological Oxygen Demand and Chemical Oxygen Demand. It also contained more quantities of chloride, sulphate, etc. The similar finding was also reported in rice mill effluent[10].

Biochemical Analysis:

Carbohydrates:

Similar trends were recorded in groundnut and paddy treated with sugar mill effluent[18], and ragi treated with sugar mill effluent[7]. The increase in the amount of soluble and reducing sugars might be either due to inhibition in starch synthesis from hexose or stimulation of starch hydrolysis[1].

Total protein:

Protein is one of the reserve food materials, which are utilized for the seedling growth. The quantity of protein in different organs of the plants showed variations. Shoot contained more protein than that of root. The protein content in root and shoot of plants increased up to 100 ppm concentration of the effluent and decreased in the further higher concentrations. Lakshmi and Sundaramoorthy[7] reported the same trend while working in ragi treatment with sugar mill effluent in and *Lycopersicum esculentum* treated with tannery effluent[13] .

Amino Acids:

Amino acid is the monomer of protein, which of the common reserve food material manufactured by plants. Shoot contained more amino acid content than that of the root. The amino acid content showed an increasing trend over control up to 100 ppm concentration of the effluent and decreased in the presence of further higher concentration. The similar observation was also recorded in paddy and groundnut, treated with sugar mill effluent[18]. Sundaram[16] reported that the amino acid content was increased in the tissues when more nitrogen was supplied to the plants. He also reported that the presence of increased amount of asparagines, which is responsible for the reduction of toxicity, was due to the accumulation of more ammonium ions.

Chlorophyll Pigments Contents:

Chlorophylls are the most important group of photosynthetic pigments responsible for light absorption and are found in the thylakoids of the chloroplasts. They contain a porphyrin ring with a magnesium ion in the centre of the molecule and a long hydrophobic tail that anchors them in the membrane. It has also been shown that the level of chlorophyll is increased in young expanding leaves and decreased substantially during senescence[6]. Chlorophyll estimation is one of the important plant parameters which are used as an index of production capacity of the plant. The chlorophyll content is an ecological index as well as growth parameters[3]. The chlorophyll content is positively correlated with net photosynthetic rate, and hence it plays a major role in controlling grain growth and grain filling process. Pandey and Rao[7] and There is also reduction in chlorophyll content as a result of pollution. The pigment contents showed an increasing trend over control up to 100 ppm concentration of the effluent and decreased gradually in further higher concentrations. The same trend was observed in Groundnut and paddy[19] in response to the treatment of sugar mill effluent and *Vigna mungo* in relation to distillery effluent treatment[11] and rice in relation to chlor alkali factory solid waste treatment[12].

Carotenoid:

The carotenoids act as accessory pigment in photosynthesis. The carotenoid content increased gradually up to 45 days. The plants treated with 100 ppm effluent concentration showed maximum carotenoid content and then decreased gradually with increase in the concentration of effluent from 100 ppm onwards. The same trend was observed in ragi[17] and in relation groundnut and paddy[19] to sugar mill effluent and tannery effluent treatments. The increase in carotenoid content at low concentrations of the effluent treatment may be due to the beneficial effect of nitrogen and other inorganic elements on carotenoid synthesis[5,15]. The decrease in carotenoid content may be either because of its degradation or inhibition of its biosynthesis by the toxic substances present in the higher

Table 1. Physicochemical characteristics of rice mill effluent with their maximum permissible limits as recommended by ISI

Physico-chemical parameters of rice mill effluent			
Parameters	**Mean value**	**ISI limit for discharge of industrial effluents**	
		On land for irrigation (ISI, 1977)	**Into inland surface waters (ISI 1974)**
Colour	Pale yellow	-	-
Odour	Unpleasant	-	-
Temperature(^{0}C)	34.46	-	-
pH	7.51	5.5 - 9.0	5.5 - 9
Conductivity (ms/s)	1.705	-	-
TSS (mg L-1)	536.66	100	100
TDS (mg L-1)	562.63	2100	2100
TS (mg L-1)	1099	-	-
Nitrate (mg L-1)	0.76	-	-
Phosphate (mg L-1)	18.1	-	-
Sulphate (mg L-1)	37.66	1000	1000
DO (mg L-1)	1.2	-	-
BOD (mg L-1)	313.7	100	30
COD (mg L-1)	513.1	-	-
Total Hardnss (mg L-1)	101.4	-	-
Total Alkalinity(mg L-1)	164.56	-	-
Chloride (mg L-1)	121.5	600	1000

Table 2. Effect of rice mill effluent on seed vigour index (SVI) of *Lycopersicon esculentum* Mill. in pot culture

Concentration	Days		
	15th day	30th day	45th day
Control	275.4	1004.4	1775.7
50 ppm	303.6	1054.32	1882.32
100 ppm	308	1017.28	1821.6
200 ppm	234.52	888.06	1571.12

Fig 1. Effect of rice mill effluent on lateral root length (cm) of *Lycopersicon Esculentum*

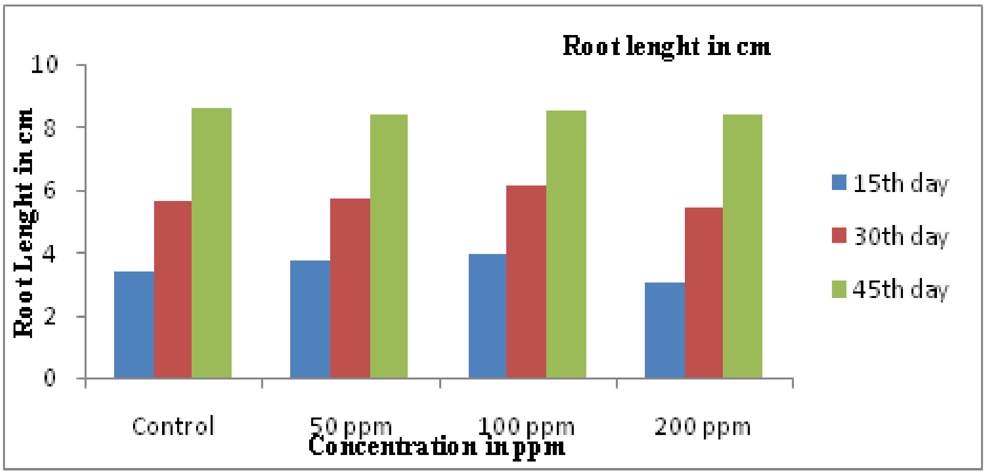


Fig 2. Effect of rice mill effluent on seedling length (cm) of *Lycopersicon esculentum*

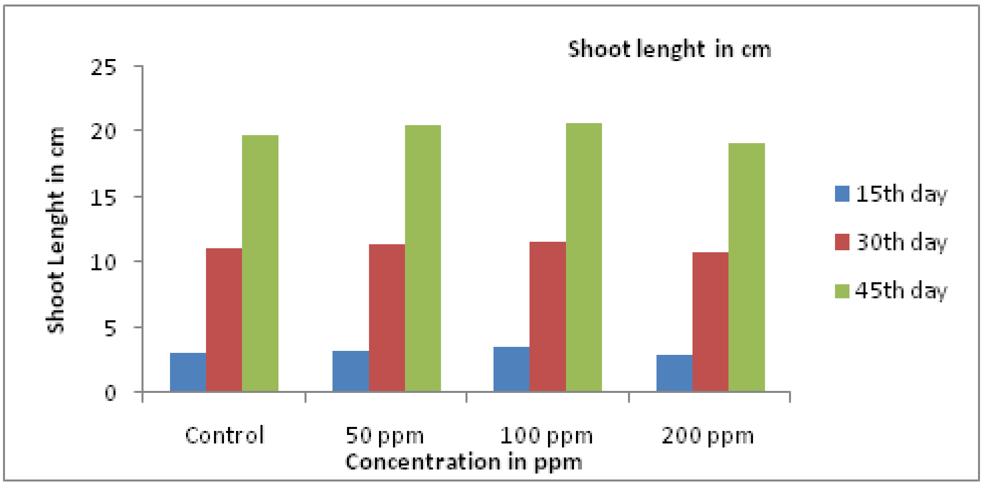


Fig 3. The carbohydrate content (mg/g fr.wt.) in the root of *Lycopersicon esculentum* treated with different concentration of rice mill effluent

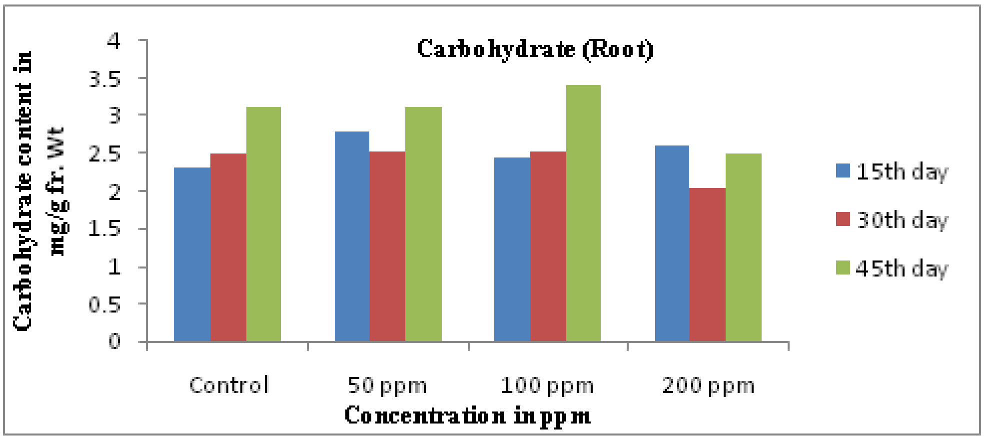


Fig 4. The carbohydrate content (mg/g fr.wt.) in the shoot of *Lycopersicon esculentum* treated with different concentration of rice mill effluent

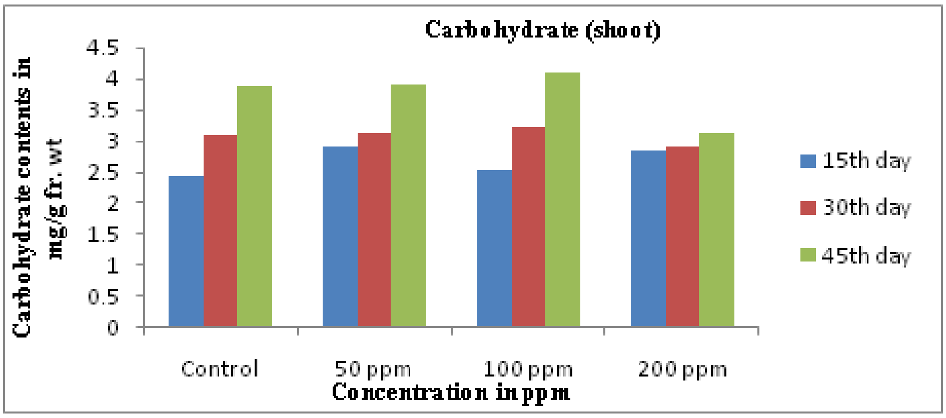


Fig 5. The protein content (mg/g fr.wt.) in the root of *Lycopersicon esculentum* treated with different concentration of rice mill effluent

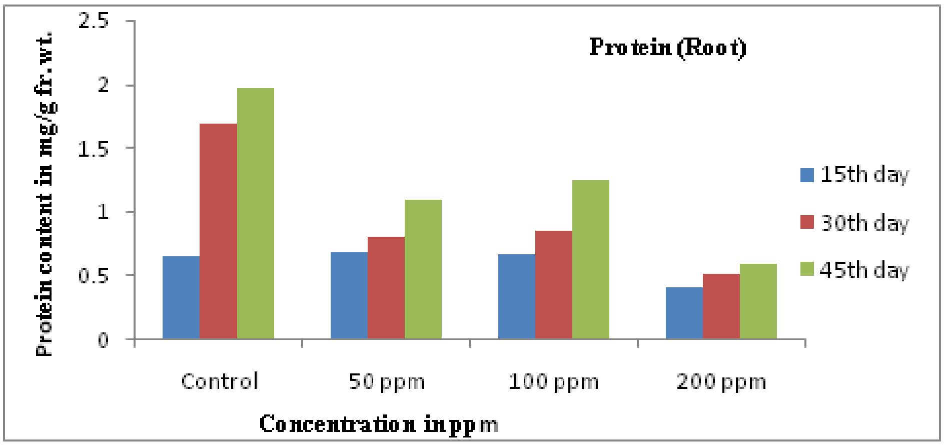


Fig 6. The protein content (mg/gm fr.wt.) in the shoot of *Lycopersicon esculentum* treated with different concentration of rice mill effluent

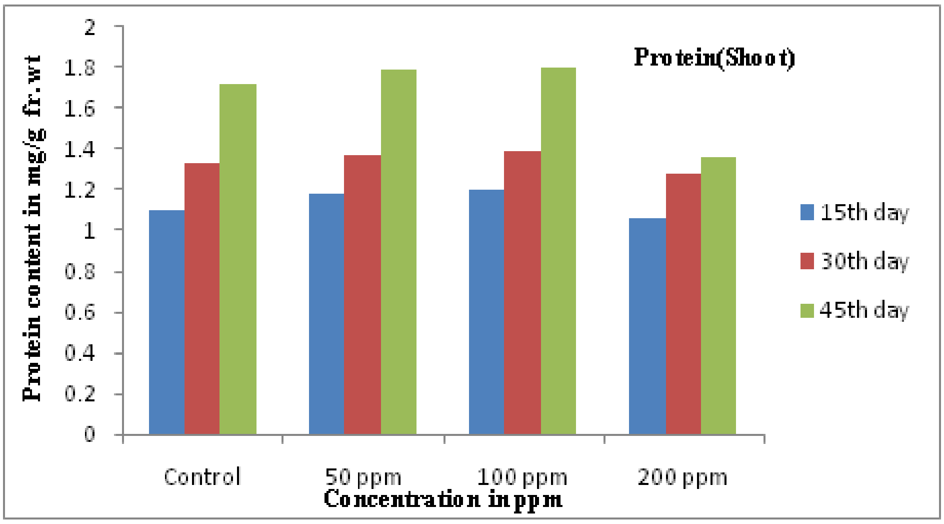


Fig 7. The amino acid content (mg/g fr.wt.) in the root of *Lycopersico esculentum* treated with different concentration of rice mill effluent

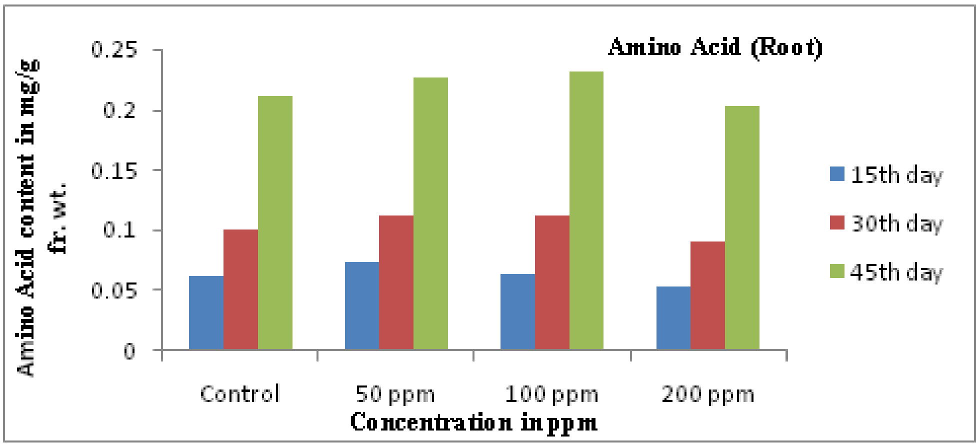


Fig 8. The amino acid content (mg/g fr.wt.) in the shoot of *Lycopersicon esculentum* treated with different concentration of rice mill effluent

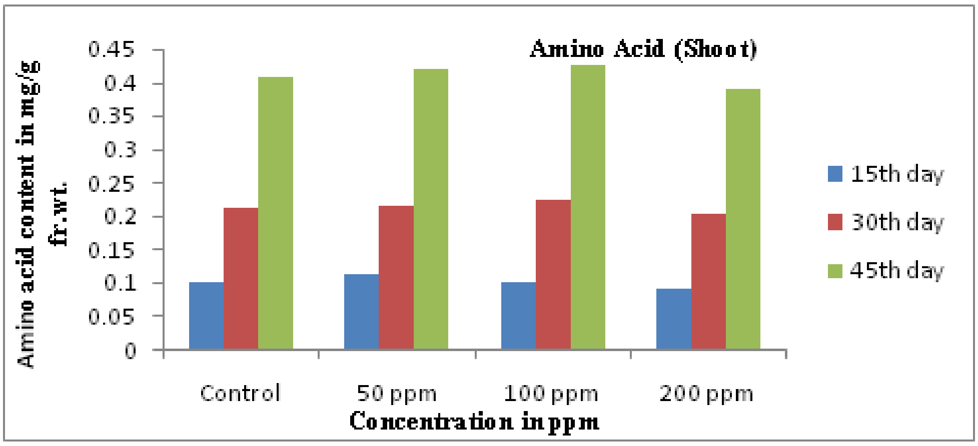


Fig 9. Effect of rice mill effluent on chlorophyll 'a 'content (mg/g) of *Lycopersicon esculentum*

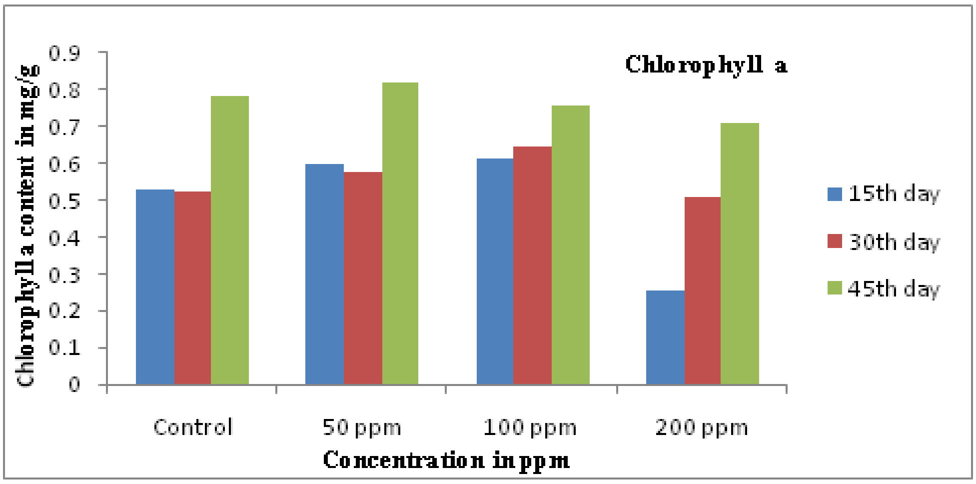


Fig 10. Effect of rice mill effluent on chlorophyll 'b' content (mg/g) of *Lycopersicon esculentum*

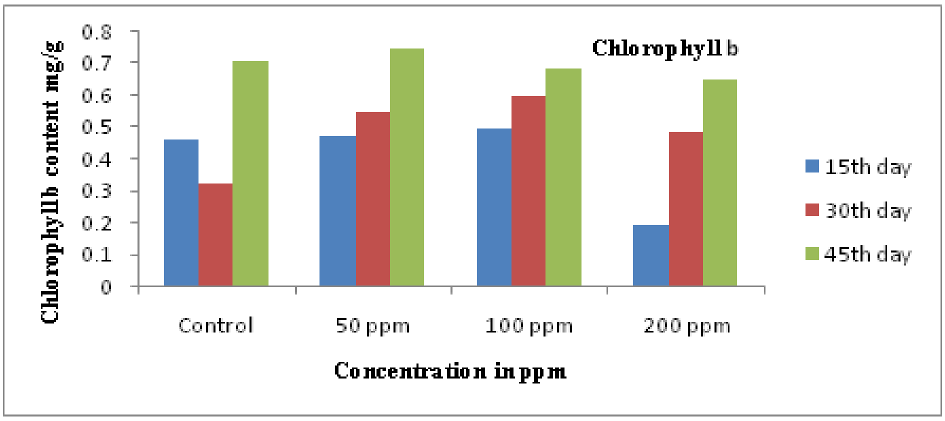


Fig 11. Effect of rice mill effluent on total chlorophyll content (mg/g) of *Lycopersicon esculentum*

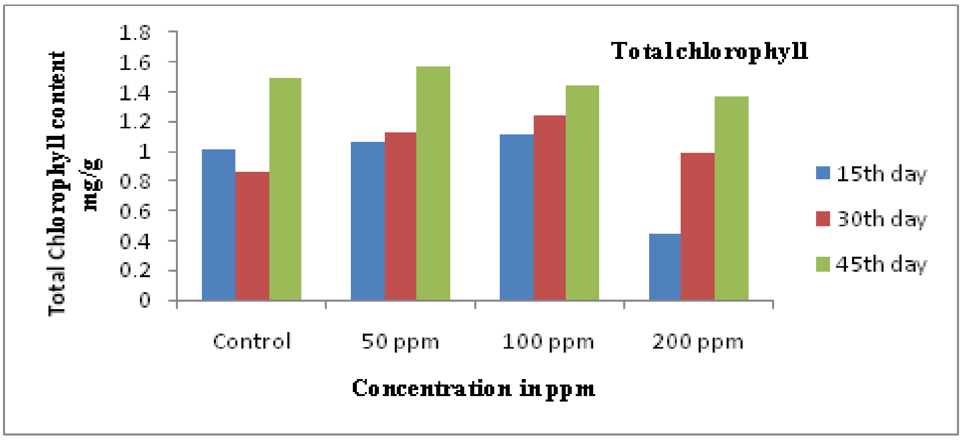
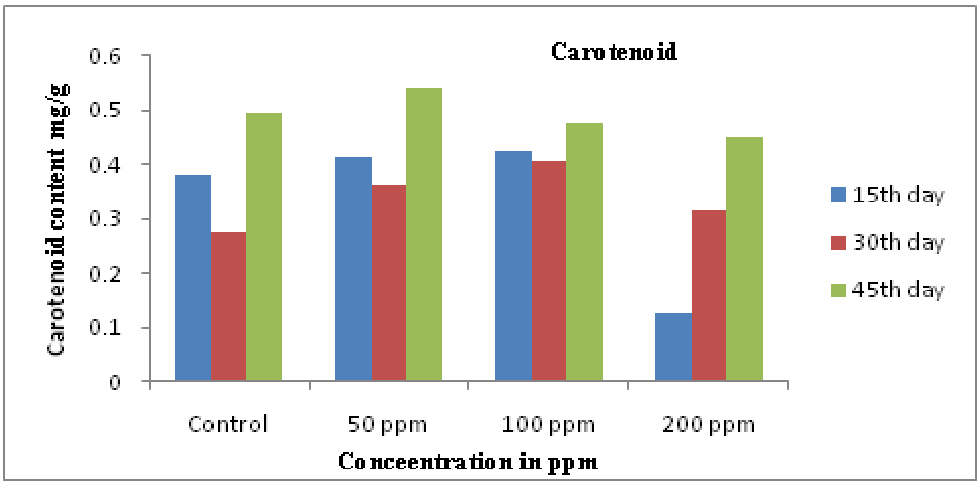


Fig 12. Effect of rice mill effluent on carotenoid content (mg/g) of *Lycopersicon esculentum*



CONCLUSION

The present study concluded that the higher concentration of effluent exert more stress on the germination potential of tomato plant where as the lower concentration effluent enhance the plant germination rate. The rice mill effluent content high amount of nutrient such as sodium & Potassium at desired concentration which enhance the plant growth potential. So, the lower concentration of rice mill effluent can be amended with the soil not only to gain better yield of crops but also to reduce the disposal problems relating to rice mill effluent.

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