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# Study of sugar mill effluent and its impact on sunflower seed germination and growth (Helianthus annuus L.)

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***Abstract***
*The sugar industry is a significant agricultural sector in India, and it contributes significantly to water pollution by discharging a lot of wastewater into bodies of water, harming plants and other living things. The impact of various concentrations (control, 10, 25, 50, 75, and 100%) of sugar mill effluent on the seed germination behaviour of sunflower (Helianthus annuus L.) was examined in the current experiment, which included physico-chemical investigations of the effluent from the E.I.D. Parry Sugar Mill. The morphological parameters including seed vigour index, tolerance index, percentage of phytotoxicity, shoot length, root length, fresh weight, and dry weight of seedlings were calculated. The findings of the sugar mill effluent studies revealed some parameters such as PH, EC, acidity, TDS, TS, BOD, COD, sulphate, magnesium, nitrogen, zinc, iron, and copper. lead, manganese and oil and grease exceeded the permissible limit compared to Tamil Nadu Pollution Control Board (TNPCB) and then germination and growth parameters increased in lower (10%) concentration of sugar mill effluent and this morphological parameters gradually decreased with increasing effluent concentration. Sugar mill effluent with a lower concentration (10%) can be applied for irrigation.*

***Keywords***
*Sunflower, Germination, Physico- chemical analysis Phytotoxicity and sugar mill effluent*

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## I. Introduction

Environmental deterioration is one of the most serious issues confronting the globe today. Industrialization is the primary cause of environmental pollution. While it is critical for developing countries, it also emits hazardous components into the environment. Industry has a significant impact on water because of the large amounts of wastewater poured into bodies of water. It is harmful to plants and other living things, and it affects the physicochemical properties of water. In reality, garbage generated by numerous human activities, especially industrial waste has become one of the leading sources of water body contamination. Human-induced activities can alter the natural source of contaminants and also initiate pollution load in the receiving water bodies (Whittemore et al. 1989). The corrosion of groundwater quality affects its usage for domestic, agriculture and industrial activities (Brindha and Elango 2012; Selvakumar et al. 2014). Approximately, one-third of death and 80% of diseases in the developing nations are caused by the drinking of contaminated water (WHO 2004). Industries are categorized into three types such as Red (high polluting industry), Orange (polluting industry) and Green (moderately or non-polluting industry) based on produced hazardous wastes into environment by Ministry of Environment and Forests, India. Industries are huge amount of waste water release into nearby water bodies and it affects the nature of water (Sundaramoorthy et al. 2006). Sugar mill is coming under red category and is one of the vital agro-based industries in India and is notably answerable for creating substantial impact on rural economic system but it released large amount of effluents at some stages in sugar manufacturing. It contained excessive degree of organic pollution and affected environment (Saranraj and Stella 2012). The enormous quantity of effluent release into water bodies with partially or without any treatment from sugar industry (Borole and Patil 2004). Nowadays, the discharged effluent is one of the main problems to be faced in the future with increased disagreeable effects (Moazzam et al. 2012). It has also been reported that high amount of pollutants present in the sugar mill effluent. It changed the normal water condition and caused adverse effects on living organisms (Ayyasamy et al. 2008).Agricultural land (sq. km) in India was reported at 1790451 sq. Km in 2020, according to the World Bank collection of development indicators, compiled from officially recognized sources. India - Agricultural land (sq. km) - actual values, historical data, forecasts and projections were sourced from the World Bank on March of 2023.Water is essential for agriculture and all living organisms. A large amount of water is utilized for irrigation, yet water scarcity has resulted in increased water demands. As a result, farmers used waste water for irrigation, which is an alternative source of water scarcity. Sugar mill produced large amount of wastewater during sugar production and discharged into nearby water bodies (Ezhilvannan et al. 2011). Sugar mill effluent had more quantity of BOD, COD, dissolved solids and suspended solids. Further to that, the excessive amount of chlorides, sulfates, nitrates, calcium, magnesium and some traceable amount of heavy metals inclusive of zinc, copper and lead were presented in this effluent that effluent used for the irrigation of surrounding farmers. The effluent harm full affects the plant growth as well as soil fertility while used for irrigation (Kumar and Chopra 2010; Jagannathan et al. 2014). Soil was highly contaminated when untreated effluent was used for irrigation which contained high organic compounds and heavy metals (Salem et al. 2000; Sebastiani et al. 2004; Singh and Bhati 2005). This effluent contains high quantity of nutrients that have the potential for use in agriculture. So, the effluent reduces the fertilizer and scar- city of water and it includes wealthy in numerous plant nutrients (Kumar and Chopra 2012).Sunflower oil is the non-volatile oil compressed from sunflower (*Helianthus annuus*) seeds. Sunflower oil is commonly used in food as frying oil, and in cosmetic formulations as an emollient. The world's largest sunflower oil producers now are Russia, Ukraine and Argentina. One of the primary reasons for the growing popularity of sunflower oil is its impressive fatty acid content and has oleic acid (omega-9) and linoleic acid (omega-6) which are the predominant monounsaturated and polyunsaturated fats (Skorić *et al*. 2008).

Sunflower *(Helianthus annuus L.)* belongs to family Asteraceae is commonly cultivated in throughout the India. Sunflower flowers are very attractive colour in nature. These fatty acids reduce the LDL cholesterol and total cholesterol, decreasing the chances of coronary artery diseases (Chowdhury *et al*. 2007). Phytosterols have been found in high amounts (270-289 mg/100gm) in sunflower oil which are efficient in reducing cholesterol, increasing immunity and reducing the risk of colon cancer (Phillips *et al*. 2005).Seed germination is a critical stage that ensures reproduction and controls the dynamics of plant populations. So, it is an important test of probable crop productivity (Radosevich et al. 1997). The analysis of sugar mill effluent and its impact on sunflower seed germination and growth is the focus of the current study (*Helianthus annuus L.*).

## II. Materials And Methods

**Collection of effluent**

The effluent samples were collected in plastic containers from the E.I.D Parry Sugar Mill's outlet at Nellikuppam, Taluk, Cuddalore District, Tamil Nadu, India. This sugar industry can be found at Latitude: 10.25278. The longitude is 78.9634690E. The effluent was transported to the Ecology Laboratory, Department of Botany, and refrigerated at 4 °C for future use.

**Seed collection**

Sunflower seeds were procured from Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India.

**Analysis of sugar mill effluent**

The collected sugar mill effluent sample was analysed for their various physico-chemical properties in Ecology Laboratory, Department of Botany, Annamalai University as per the routine Standard methods mentioned in APHA (2005).

**Preparation of different concentrations of effluent**

The wastewater sample taken from the sugar mill industry's outflow was processed as 100% pure raw effluent. As necessary, fresh preparations of various concentrations of sugar mill effluent10, 25, 50, 75, and 100% were made using distilled water (Lakshmi and Sundaramoorthy 2000). They were employed in germination research.

Control: Distilled water
10% : 10 ml effluent + 90 ml water
25% : 25 ml effluent + 75 ml water
50% : 50 ml effluent + 50 ml water
75% : 75 ml effluent + 25 ml water
100% : Raw effluent

**Germination studies**

The healthy and uniform sized Sunflower seeds were selected and surface sterilized with 0.1% $HgCl_2$ for 2 min and then thoroughly washed with tap water. Fifty seeds were placed equidistantly in plastic cups filled with 100 g sterilized soil (clay + sand (1:1)). The seeds were irrigated with equal quantity of different concentrations of effluent and the seeds irrigated with distilled water were treated as control. Three replicates were maintained for each treatment including control. The germination percentage, shoot length, root length, seedling fresh weight and seedling dry weight (Milner and Hughes 1968; Sundaramoorthy et al. 2006 Sajani and Muthukkaruppan 2011) were taken and recorded on the 15th day seedlings. The values of seed vigour index, Tolerance index and percentage of phytotoxicity were also calculated.

**Germination percentage**

The number of seeds germinated in each concentration was counted on the 15th day and the germination percentage was calculated using the following formula.

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total numbers of seeds sown}} \times 100$$

**Shoot and root length (cm/seedling)**

Five seedlings were taken from each treatment and their shoot length and root length were measured by using a cm scale and these values were recorded.

**Fresh weight (g/seedling)**

Five seedlings were collected from each treatment and their fresh weights were measured with the help of an electrical single pan balance.

**Dry weight (mg/seedling)**

The same seedlings used for fresh weight were kept in hot air oven at 80 °C for 24 h. Then, the seedlings were taken from the oven and kept in desiccators for some time. Their dry weights were taken using an electrical single pan balance.

**Vigour index**

Vigour index of the seedlings was calculated using the formula proposed by Abdul-Baki and Anderson (1973).

$$\text{Vigour index} = \frac{\text{Germination percentage}}{\text{Length of seedling}} \times 100$$

**Tolerance index**

Tolerance index of the seedling was calculated using formula proposed by Turner and Marshal (1972).

$$\text{Tolerance index} = \frac{\text{Mean length of longest root in treatment}}{\text{Mean length of longest root in control}} \times 100$$

**Percentage of phytotoxicity**

The percentage of phytotoxicity of effluent was calculated using the formula proposed by Chou et al. (1978).

$$\text{Percentage of phytotoxicity} = \frac{\text{Radicle length of control- Radicle length of test}}{\text{Mean length of longest root in control}} \times 100$$

**Statistical analysis**

The experiment was repeated three times and data recorded each time were assembled for statistical analysis to deter- mine the significance of variance (P \ 0.05). For comparison of treatment means, standard errors were computed using SPSS (16.0) and Microsoft Excel programme.

## III. Results and discussion

Physico chemical analyses of sugar mill effluent are given in Table 1.The evaluation of sugar mill effluent confirmed that it is acidic in nature and dull white in colour with decaying molasses smell. Colour is vital role of an aquatic ecosystem and it affects photosynthesis. Colouration reduced the some other parameters such as temperature DO and BOD, etc., and it also reduced to the decomposition of substances by microbes (Ingaramo et al. 2009; Buvaneswari et al. 2013; Saurabh and Shailja 2014). Decomposition of organic matter under anaerobic condition produced various sulphides, particularly ferrous sulphide which is caused for colour of the effluent. This is the most common gas and it simply soluble in water, colourless and inflammable, however, highly toxic. Some other gases, such as carbon dioxide, nitrogen, etc., from decomposition of organic compounds are responsible for odour of effluent. This is in conformity with the earlier finding of Vijayaragavan et al. (2011) and Rathore et al. (2000). In the present investigation, the pH value of the sugar factory effluents was 4.04, which is acidic in nature. pH is very important factor for ecosystems that serve as an index for pollution.Itis an indicator for the sustainability for the aquatic organ- isms. The normal pH is changed in the ecosystem which can affect living organisms. Various ions are present in the effluent and it directly related with pH of the effluent. The reaction among effluent flowing from open drainage system with the soil has direct relevance to pH of the effluent. The pH was acidic in nature because of the use of phosphoric acid and sulphuric acid during the clarification of sugar- cane juice (Ayyasamy 2008). The sugar mill effluent contain high level of EC (4745 Mm/h) which has a harmful affect to living organisms of the ecosystem. The temperature plays a major role in aquatic environment which is very high in sugar mill effluent (36 °C) that has a lethal effect on the diversity of the aquatic environment. Normally, the organisms present in aquatic conditions rapidly grow at a temperature in the range of 20–27 °C (Ezhilvannan et al. 2011). The increased temperature may accelerate the rate of chemical reaction and chemical changes in the aquatic condition (Shiva Kumar andSrikantaswamy 2015). In agriculture irrigation has agree able limits of temperature are 40 °C. So, this sugar mill effluent has suitable temperature for irrigation It contained considerable amounts of SS (180 mg/l), chloride (314 mg/l), fluoride (1.88 mg/l), calcium (124.8 mg/l). Suspended particles are present in the water bodies which affect the light intensity of aquatic and it impact the turbidity and transparency of water bodies. The TDS and TS of sugar mill effluent were 3725 and 3905mg.The findings were also in accordance to Borole and Patil (2004, Vinod and Chopra (2014a, b). Dissolved solids were in the form of colloidal substances which dissolved in effluent. The rate of dissolved colliding particles is referred to collision and the pH affected dissolved rate of collision in the effluent. Dissolved and non-dissolved substances called as total solids and it composed of carbonates bicarbonates, chlorides, sulphates, nitrates, Ca, Mg, Mn, organic matter, silts and other particles which caused pollution of water bodies. It affects the intensity of light and living organisms (Poddar and Sahu 2015). BOD is an important parameter that indicates the magnitude of water pollution, by the oxidizable organic matter and the oxygen used to oxidize inorganic material such sulphides and ferrous ions. Present investigation showed that the effluent hashigh value of BOD (3480 mg/l) and COD (7880 mg/l). Thechemical kinetic factor like temperature, pressure can affect the BOD reaction. BOD indicates of water pollution caused by oxidation of organic substances. It is one of the valuable parameter of the water quality. It is clear from the data that COD of effluent exceed the TNPCB limit. The high COD value is because of the presence of excessive amount of organic wastes. Saranraj and Stella (2012) analysed various parameters of sugar mill effluent and they have recorded the high COD value. The COD test determines the required amount of oxygen for oxidation of organic substances by chemical oxidant. The strong oxidizing agents should be oxidized completely all organic substances except some other substances of effluent in acidic condition. The BOD and COD tests are used in indication of toxic conditions and the presence of biologically resistance substances in the sugar mill effluent (Malik et al. 2014; Poddar and Sahu 2015). High amount of magnesium (286 mg/l), sulphate (290.88 mg/l), nitrogen (1250 mg/l), oil and grease (19 mg/l) and the toxic heavy metals such as zinc (0.89 mg/l), iron (16 mg/l), copper (0.420 mg/l), lead (0.52 mg/l) and manganese (0.068 mg/l) were recorded in the collected sugar mill effluent sample. Besides the metals, oil and grease were used in sugar mill for various processes. It influenced the temperature, pH, BOD, COD, DS and TS of the effluent. It affected all living organisms of aquatic and terrestrial ecosystems. Similar findings were recorded and reported by Rathore et al. (2000), Lakshmi and Sundaramoorthy (2000), Borole and Patil (2004).Seed germination and seedling growth are vital for continuation of plant life and they are extremely vulnerable to environmental stress. Since germination is the first physiological process, several growth parameters such as percentage of germination and ultimately growth and yield of the crops are taken as criteria to assess the degree of pollution (Mishra and Pandey 2002). In the present investigation, the effects of different concentrations of sugar mill effluent on seed germination of

Sunflower (*Helianthus annuus L.*) were reported. The highest values of seed germination percentage were recorded at 10% of sugar mill effluent concentration and the lowest values of germination percentage were recorded at 100% of sugar mill effluent concentration (Fig. 1). The increase in germination percentage over control at lower concentrations (10%) indicates the stimulation of physiologically inactive seeds of the lot by the effluent treatment as reported by Vinod (2014), Vaithiyanathan et al. (2014) and Suresh et al. (2014). The favourable amount of nutrients may be presented in lower concentration of sugar mill effluent. It created good environmental condition for seed germination then the seed of the nutrient present in the effluent. The lower concentration of effluent had many nutrients such as nitrogen, phosphorous, etc. which might have promoted the plant growth as suggested by Augusthy and Annsherin (2001). At the same time, the higher concentrations of sugar mill effluent inhibited the germination of Sunflower. Large amount of organic and inorganic substances presented in higher concentration of sugar mill effluent which adversely affected the seed germination because of the higher salt content which caused change of osmotic pressure outside of the seed. It decreased water absorption of the seed and then inhibited the seed germination (Adriano et al. 1973).

The highest shoot length root length (6.54 cm/seedling), fresh weight and dry weight were observed in lower concentration (10%) of sugar mill effluent and the lowest shoot length, root length ,fresh weight and dry weight were observed in higher concentration (100%) of sugar mill effluent (Figs. 2,3and 4). The highest vigour index and tolerance index of seedlings were observed in 10% of effluent treated seedling and lowest vigour index and tolerance index were observed in 100% of effluent (Figs. 5,6and7). Percentage of phytotoxicity level was high (0.65%) at 100% of effluent treated seedling (Fig. 8). Seedling growth and development are essential processed of life and propagation of plant species. They continuously depend on the external environment. Presence of various pollutants in lower concentrations of sugar mill effluent (at 10%) increased the growth and development. These observations are in conformity with Lakshmi and Sun daramoorthy (2000), Saxena and Madan (2012), Ali et al. (2012) Malik et al. (2014). Higher concentrations of sugar mill effluent inhibited the root and shoot length of seedlings. It contained large amount of DS and SS which interfered and decreased the absorption of some other nutrients. The interference of heavy metals decreased the root and shoot length of the plant might be due to the affect of physiological processes of plant and it also involved in inhibition of enzyme activities, affected the nutrition, water imbalance and alternation of hormonal status changed the membrane permeability (Sharma and Dubey 2005). The fresh and dry weight of seedlings increased at 10% of sugar mill effluent concentration while decreased at higher concentration of sugar mill effluent. Some amount of nutrients and trace elements may be needed for seeds. These nutrients also presented in the effluent and it is important for plant growth. The lower concentration of sugar mill effluent contained required amount of nutrients presented which developed the growth of seedlings as well as fresh and dry weight of the seedlings. The required amount of various chemicals presented in the lower concentration of sugar mill effluent which promoted the plant growth (Lakshmi and Sundaramoorthy 2000; Siva Santhi and Suja pandian 2012). The higher concentrations of sugar mill effluent minimized the fresh weight and dry weight of seedlings. The decrease of seedlings weight may be due to the poor growth of seedlings under the higher concentrations of effluent irrigation.

**Table: 1 Physico chemical properties of sugar mill effluent**

| S.No | Properties | Raw effluent | Tolerance limits |
|---|---|---|---|
| 1. | colour | Dull white | Colourless |
| 2. | odor | Decaying | - |
| 3. | pH | 4.04 | 5.5–9.0 |
| 4. | Electrical | 4744 Mm/h | – |
| 5. | conductivity (EC) Temperature (°C) | 36.0 | 40.0 |
| 6. | Acidity | 1340.0 | – |
| 7. | Suspended solids | 180.0 | 200 |
| 8. | Total dissolved solids | 3725.0 | 200 |
| 9. | Total solids | 3905.0 | 2100 |
| 10. | BOD | 3480.0 | 30 |
| 11. | COD | 7880.0 | 250 |
| 12. | Chloride | 314.0 | 600 |
| 13. | Sulphate | 290.88 | 12 |
| 14. | Magnesium | 286.0 | 100 |
| 15. | Phosphorous | 7.2 | 10 |
| 16. | Nitrogen | 1250 | 600 |
| 17. | Fluoride | 1.88 | 1.0 |
| 18. | Silica | 99.0 | – |
| 19. | Calcium | 124.8 | 200 |
| 20. | Zinc | 0.89 | 0.01 |

| | | | |
|---|---|---|---|
| **21.** | **Iron** | **16.00** | **1.00** |
| **22.** | **Copper** | **0.420** | **0.01** |
| **23.** | **Lead** | **0.52** | **0.05** |
| **24.** | **Manganese** | **0.068** | **0.01** |
| **25.** | **Oil and grease** | **19** | **10** |

**Fig: 1 Germination percentage of Sunflower (*Helianthus annuus L*) under grown different concentrations of sugar mill effluent**

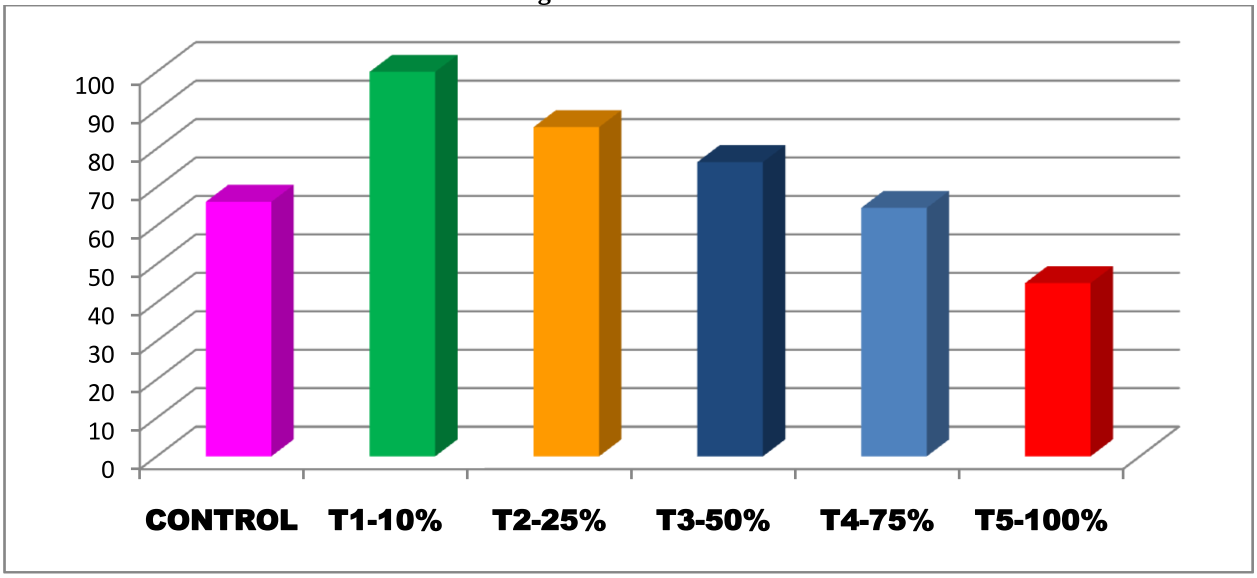



**Fig: 2 Shoot Length of Sunflower (*Helianthus annuus L*) under grown different concentrations of sugar mill effluent**

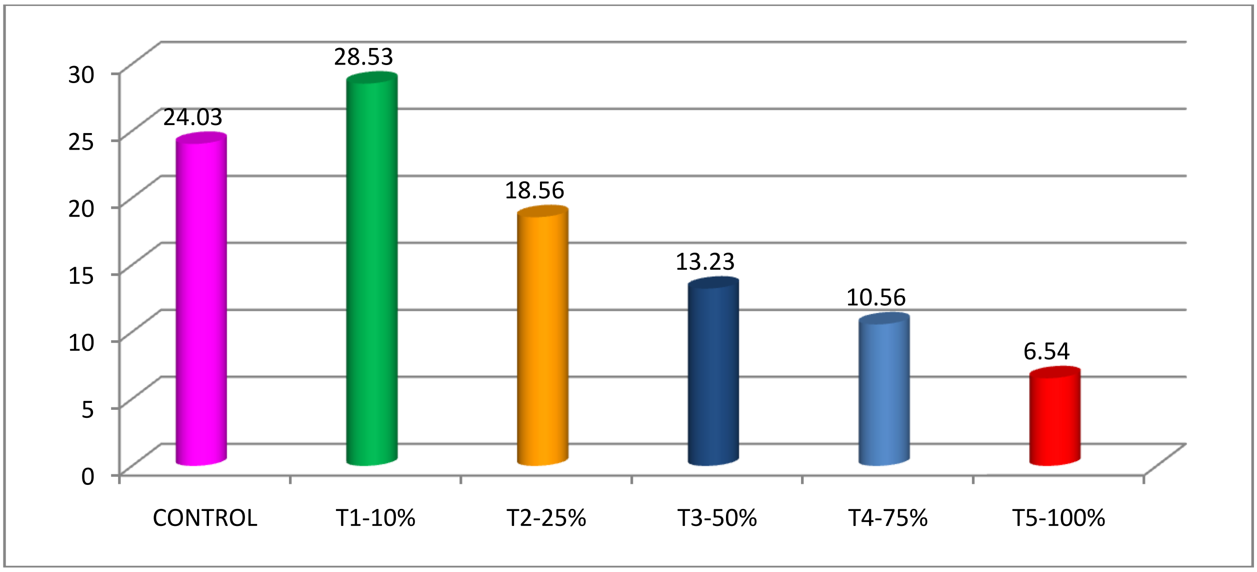


**Fig: 3 Root Length of Sunflower (*Helianthus annuus L*) under grown different concentrations of sugar mill effluent**

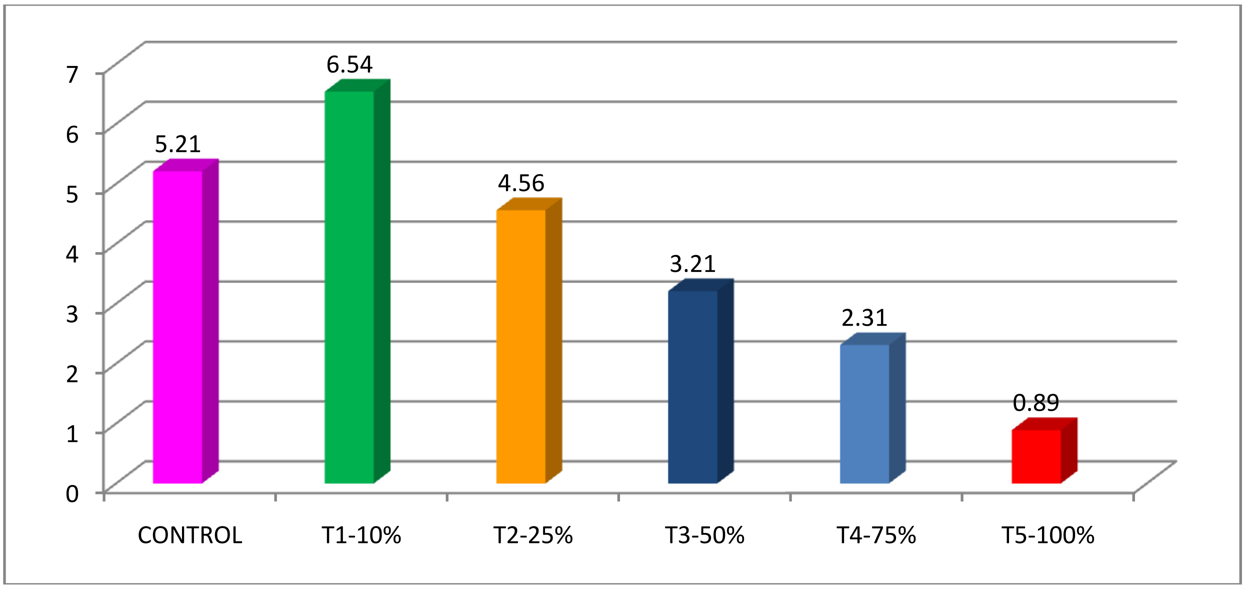



**Fig: 4 Fresh weight (mg\Seedlings) of Sunflower (*Helianthus annuus L*) under grown different concentrations of sugar mill effluent**

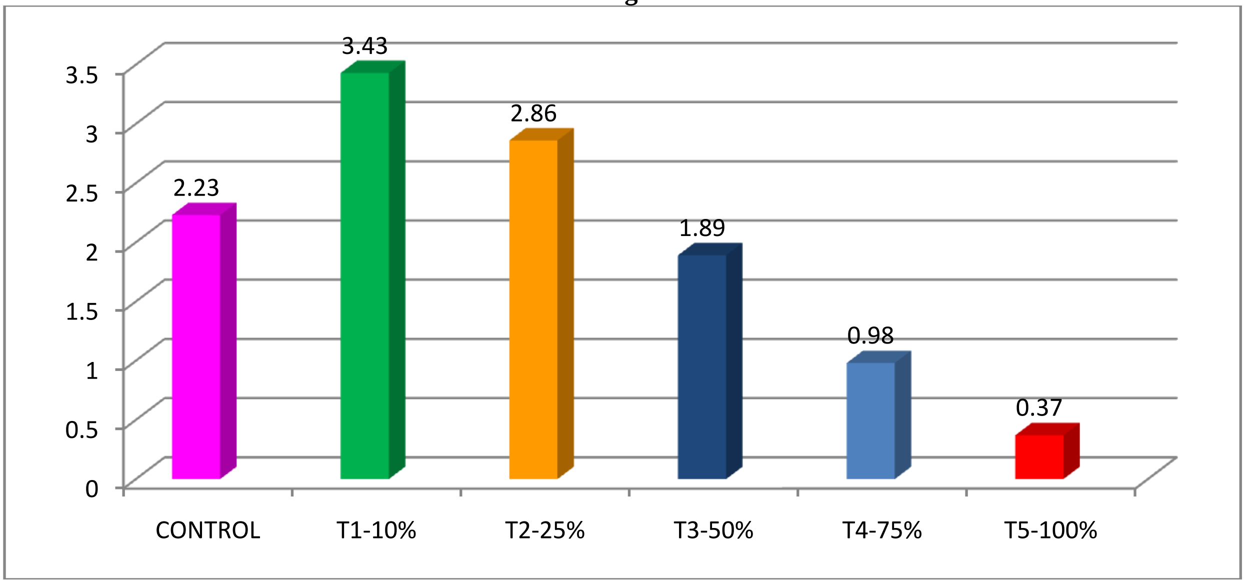


**Fig: 5 Dry weight of Sunflower (*Helianthus annuus L*) under grown different concentrations of sugar mill effluent**

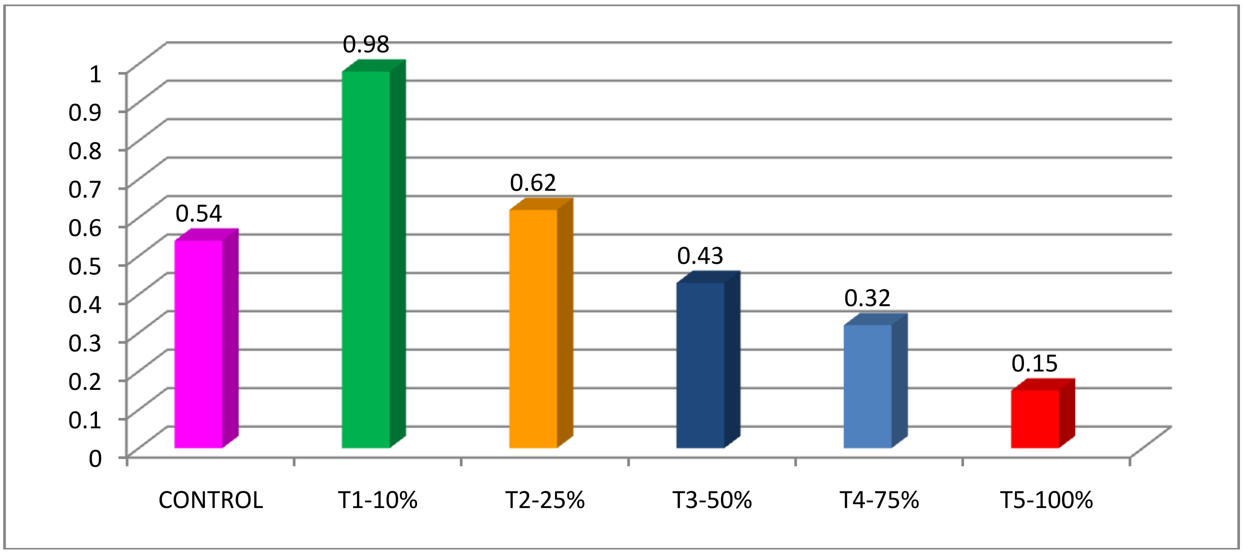



**Fig: 6 Vigour index of Sunflower (*Helianthus annuus L*) under grown different concentrations of sugar mill effluent**

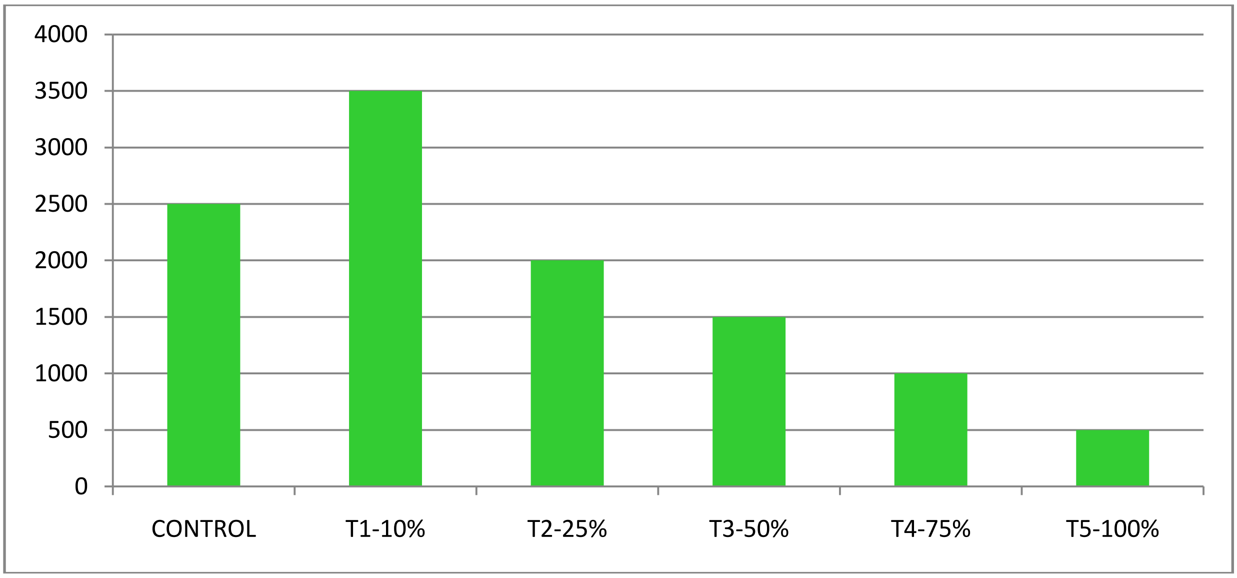


**Fig: 7 Tolerance index of Sunflower (*Helianthus annuus L*) under grown different concentrations of sugar mill effluent**

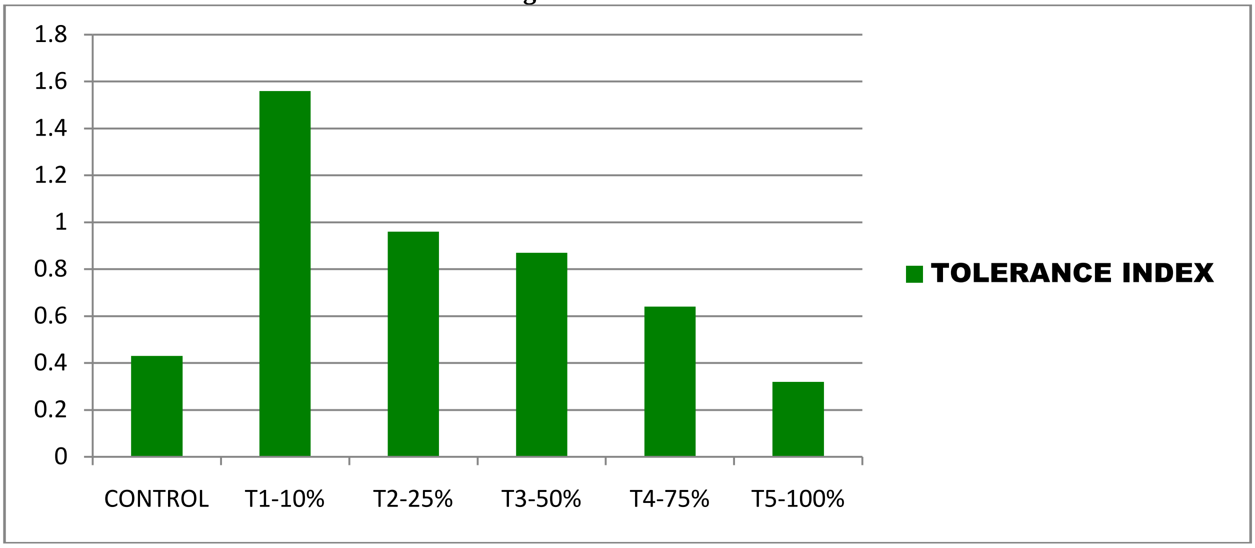
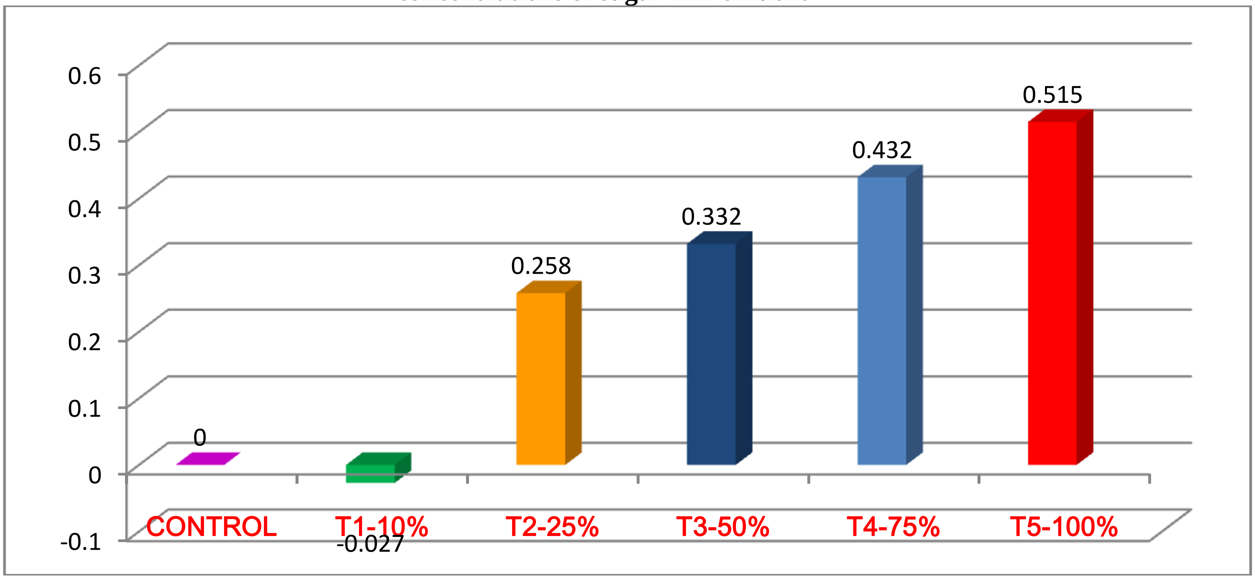



**Fig: 8 Percentage of Phytotoxicity of Sunflower (*Helianthus annuus L*) seedlings under grown different concentrations of sugar mill effluent**

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## IV. Conclusion

According to the findings of the current study, the sugar mill effluent contained more organic and inorganic chemicals than was acceptable for irrigation of agricultural land. Sunflower (*Helianthus annuus L.)* seed germination and seedling growth were stimulated by a sugar mill effluent concentration of 10% compared to other concentrations and control, and these effects gradually diminished as effluent concentrations increased. Moreover, it may be deduced that increased nutrients can also be hazardous and limit seed germination and seedling growth in addition to toxic metals. As a result, sugar mill effluent needs to be diluted in order to reduce its toxicity and because it has the potential to be used as natural fertilizer. Also, following appropriate care and dilution, the lower concentration of effluent can be used for agricultural irrigation.

**Abbreviations**

APHA American Public Health Association
BOD Biological oxygen demand
COD Chemical oxygen demand
DAS Days after sowing
DO Dissolved oxygen
EC Electrical conductivity
G % Germination percentage
HgCl2 Mercury chloride
mM/hrs Millimole per hours
ppm Parts per million
SS Suspended solids
TDS Total dissolved solids
TNPCB Tamil Nadu Pollution Control Board
TS Total solids

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