Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 2103 – 2115
Received 05 March 2021; Accepted 01 April 2021.

Performance of Germination, Development, Yield, and Pigment on Irrigation with Untreated Paint Industry Wastewateron*tagetes Erecta* L. Var Pusa Basanti

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Abstract

Tagetes erecta L. var Pusa basanti was used as the test plant to estimate the irrigation-potential of paint industry wastewater taken from Bergers Paint Private Limited situated at State Industrial Development Corporation, Samba, Jammu and Kashmir (SIDCO). The dilutions were prepared at T_0, T_{20}, T_{40}, T_{60}, T_{80}, and T_{100} (concentrations ranging from 20% to 100%). The aim was to see the performance of germination, growth, yield, and pigment content at various concentrations. The parameters were found out to be maximum at the lowest concentration (T_{20}) with maximum dilution of 80%. Germination percentage was estimated to be maximum (41.6%). The positive parameters gave the highest values at 20% concentration (T_{20}), and values then declined giving the lowest value at 100% (T_{100}), and vice versa were obtained for the negative parameters. Growth parameters included length of root, shoot, root-shoot ratio, number of leaves, plant height, dry and fresh weight. These parametersindicated the peak values at lower concentrations and vice-versa was obtained at highest concentration. The economic output increased at initial levels upto 60% and then declined. Stover yield though increased at T_{20} but further at higher concentrations, it declined, and the harvest index was highest at T_{100}. The study revealed that lower wastewater concentration caused a stimulatory effect in chlorophyll a and b, whereas total chlorophyll was significant at higher concentrations. Total chlorophyll increased up to 60% concentration but decreased at a higher concentration as the number of days after sowing (DAS) increased. Correlation and ANOVA were performed, which clearly described that the various parameters showed a declining trend with the rising wastewater concentration.

Key words: Carotenoids, Chlorophyll, Germination, Growth, Harvest index, Stover yield, Wastewater

Introduction

With nearly one percent of water resources available for human consumption, water still holds one of the highly plentiful natural resources on the planet (Grey et al., 2013;Kamboj and Choudhury 2013; Anjum et al., 2016; Tian et al., 2018;Choudhury et al., 2021). Throughout the world, water management systems are facing the huge challenge of ever-increasing insecurity of water, floods, and contamination thereof. As per the United Nations Organization estimates, about 80% of sewage is presently released without any prior treatment [UN WWAP (United Nations World Water Assessment Programme), 2017]. In India, industrial wastewater treatment, particularly in the urban and suburban zones, has been a huge challenge. In its report, the Central Pollution Control Board (CPCB) 2013, in

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its report, declared that 19,827 MLD out of 53,998 MLD generated were treated in metropolitan, class one, and class two cities.

Further, it was stated that in 2017, out of 18.6% of total treatment capacity, merely 13.5% of sewage was treated (CPCB, 2013 and CPCB, 2017c). Industries are based on the use of water for many operations, which later on lead to the release of polluted water from the industries across the world (Owa et al., 2013; Kamali and Khodaparast, 2015), and the majority of them end up as industrial wastewater (Ranade and Bhandari, 2014; Banerjee et al., 2021). The release of wastewater from the industrial sector is a big concern these days which directly goes to the environment and causes toxicity problems. The paint industry is one among many such industries which is a source of environmental pollution. Paints with variety of chemical composition are used in the whole of the world daily. For example, emulsion paints are a combination of organic and inorganic pigments, extenders, latexes, cellulosic and non-cellulosic thickeners (Krithika and Ligy, 2016). Wastewater from the paint industry frequently comprises constituents that act as a precursor in the manufacturing of paints (Zak et al., 2014). These may include water from the clean-up procedures containing poisonous chemicals, remaining acids, coating metals (Chidozie and Nwakanma, 2017). These wastewaters also contain hazardous chemicals. If released into the environment without prior treatment, they may infiltrate and leach into the groundwater and pollute the groundwater (Olaoye and Oladeji, 2015; Paul et al., 2019). Ongetting discharged into the nearby water bodies, the heavy metals get mixed up in the water bodies. Due to their persistence, they become available for uptake by the aquatic life forms. Another issue is the health and related problems like cancer, deformities, the mutation in genes, and problems in the kidneys, resulting from heavy metals in the paints (Chidozie and Nwakanma, 2017). Irrigation with leftover water from industrial activity is a longstanding practice worldwide (Tripathi et al., 2011). It offers the farmer and agriculture sector water rich in nutrients for irrigation on the one hand and the social set up with a dependable and economical system where the treatment of wastewater and disposal is manageable (Feigin et al., 1991). As a nutrient source, it also acts as a cheap means of irrigation for the farmers, and hence they use industrial wastewater to irrigate their fields. It is challenging to manage industrial wastewater as it has to be used without treatment. As it is not free from toxic chemicals and therefore affects plants' growth when released directly onto the land without prior treatment.

To estimate the irrigation potential of industrial wastewater, paint industry's wastewater was used to irrigate the test plant, *Tagetes erecta* L. var. Pusa basanti (marigold). It was analyzed for germination, growth, and biochemical characteristics including chlorophyll and carotenoids under different untreated paint industry wastewater concentrations. This experiment was conducted to assess the potential of industrial wastewater to be used as a source of irrigation. To date, there is no study performed on the *Tagetes* using paint industry wastewater as irrigant. Therefore, an attempt has been done to estimate the probable effects of wastewater from the paint industry on germination parameters, growth pattern, and yield and pigment content of the *Tagetes erecta* at various concentrations.

Materials and Methods

Study area: It was an experimental work carried out in the department's laboratory at the University of Jammu, Jammu, and Kashmir. The study area lies at an altitude of 1030 feet above sea level with GPS coordinates of 740 24' and 50 18' east and 320 50' and 330 30' north. The annual precipitation of the zone is 1000m. The maximum temperature shoots up to 47^0C in summer while the minimum temperature goes down to 4^0C in winters. The

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wastewater was collected from the Berger Paints industry located in the State Industrial Development Corporation (Samba), Jammu.

Materials: Materials used for the experiment were plastic trays of 450 mm×350 mm×75 mm dimensions, earthen pots having 220 mm diameter and 5 kg capacity, and chemicals and glassware as per the requirement. Soil samples were procured from the agricultural field at Akhnoor, Jammu, which was dried under the sun. The ratio of soil and farmyard manure was at 3:1. The fungicide bavistine at the rate of 2 gL-1 of water was sprayed on the soil and mixed thoroughly, and the hybrid seeds of *Tagetes erecta*L. var. Pusa basanti.

Methodology: The experiment of germination was conducted in the BOD incubator in November. Twenty healthy seeds were selected and sown in the petridishes in triplicates. The experiment was carried out for twelve days till the germination became constant in all the petridishes, and the counting of seeds was done every second day. Estimated parameters included percentage germination, germination value, peak value, germination period, germination speed, delay index, percent inhibition, and growth and development parameters, including length of root and shoot, weight in terms of dry and fresh as well as moisture content. Germination criteria were visible emergence of the seedlings from the soil, and it was expressed in percentage.

For parameters like growth, yield, and pigment analysis, a pot-level experiment was conducted. 100 ml of each treatment with tap water (in case of control) and different wastewater concentrations (in case of treatments) were sprinkled on the soil in respective sets on alternate days. The trays were also covered with muslin cloth until some germination was observed. The saplings were then transferred in the pots on the 30th day after sowing (DAS).

Germination parameters: Different germination parameters were calculated with the help of below mentioned formulas:

a) Percentage germination: Percentage germination= $\frac{Total\ seeds\ germinated}{Total\ seeds\ sown} \times 100$

b) Germination Index: It was estimated taking following Zucconi *et al.*, 1981.

$$\text{Germination Index} = \frac{No.of\ grown\ seeds\ in\ sample}{No.of\ grown\ seeds\ in\ control} \times \frac{Average\ sum\ of\ root\ length\ in\ sample}{Average\ sum\ of\ root\ length\ on\ control} \times 100$$

c) Delay Index (DI): It was estimated for comparing performance of crop at various applications (Modified after Kaushik *et al.*, 2005).

$$DI=X/Y$$

here,
X=delay in germination time over control (upto 20%)
Y=germination time for control

On similar lines, percent inhibition, germination value, peak value, speed of germination and germination time were also calculated with the help of formulae taken from Czabator (1962).

Growth parameters

a) Root length: Three plants were uprooted at specified intervals and their root lengths were measured and they were expressed in centimeters (cms).
b) Shoot length: The shoot lengths were also measured and were expressed in centimeters (cm).
c) Plant length: Plant length was calculated and expressed in centimeters following Shakila and Usha, 2011.
d) Biomass: Biomass of the plant samples was determined by the procedure given by APHA (2005
e) Moisture content: Percent moisture was estimated as:

$$\text{Moisture content} = \frac{Fresh\ weight - Dry\ weight}{Fresh\ weight} \times 100$$

Floral characteristics: Various flower characteristics like number of flowers per plant, average flower diameter, average flower weight (fresh), and weight of seeds per treatment were also measured.

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Yield parameters: Crop yield was expressed both in terms of economic yield as weight of flowers per plant, as stover yield by subtracting economic yield from total biomass yield or straw yield and harvest index by dividing total economic yield by above ground biomass of plant.

Biochemical parameters: The amount of chlorophyll and carotenoid was calculated according to methods described by Arnon (1949) and Duxbury and Yentsch (1956), respectively.

Results

The various parameters estimated are explained below:

Germination parameters: The experiment of germination was conducted in the BOD incubator where germination was noted each day starting on 3rd day after sowing upto 12th day after sowing (DAS). The 12th DAS was the day when seed germination became constant for all the treatments **(Plate 1 & 2).**

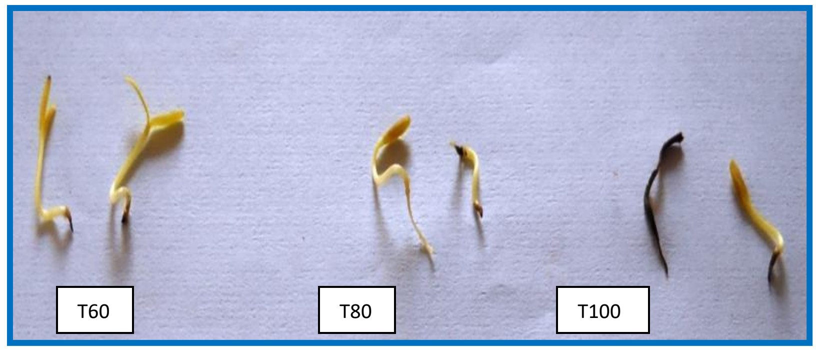

Plate 1 & 2: Uprooted plants after germination at T_0, T_{20}, T_{40}, T_{60}, T_{80} and T_{100} respectively

The maximum percentage of germination (51.6%) was revealed in the control set, T_0. At various concentrations, the use of wastewater indicated the maximum percentage germination (41.6%) at T_{20} and minimum (8.3%) at T_{100}. The trend showed that there was decreased with the increase in wastewater concentration, i.e., 41.6%, 31.6%, 30%, 11.6%, and 8.3% at T_{20}, T_{40}, T_{60}, T_{80}, and T_{100}, respectively**.** The effect of wastewater at various concentrations on different germination parameters is presented graphically in **Fig. 1 and Fig. 2**, which revealed that as the concentration of wastewater increased, the values for positive germination parameters declined, whereas negative germination parameters values increased. Delay index and percent inhibition being the negative germination parameters were zero when rinsed with tap water.

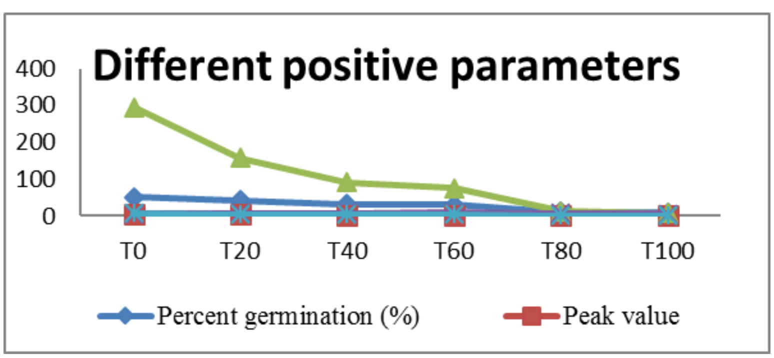

Figure 1: Positive Germination parameters at T_0 to T_{100}

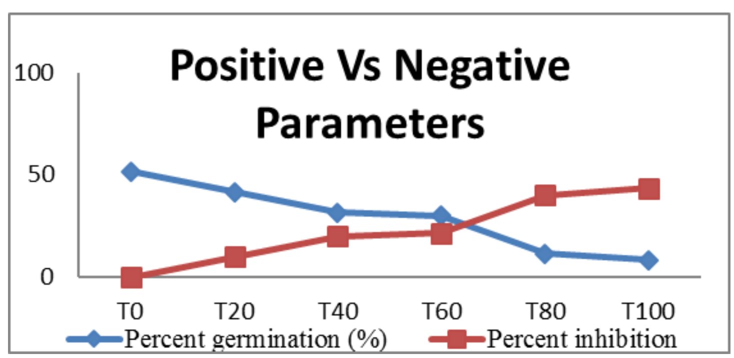

Figure 2: Positive Vs negative germinationparameters

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The comparison between the positive and negative germination parameter at various concentration level is presented in **Fig. 2**. Relation between the positive and negative germination parameters is presented using correlation coefficient below in **table 1**.

Table 1: Table showing the relation between the positive and negative germination parameters

Items	Percent germination	Speed of germination	Root length	Shoot length	Plant height	Percent inhibition
Percent germination	1.00000	0.97911	0.93314	0.94300	0.92640	-1.00000
Speed of germination	0.97911	1.00000	0.95852	0.94228	0.93917	-0.97911
Root length	0.93314	0.95852	1.00000	0.97770	0.99050	-0.93314
Shoot length	0.94300	0.94228	0.97770	1.00000	0.99465	-0.94300
Plant height	0.92640	0.93917	0.99050	0.99465	1.00000	-0.92640
Percent inhibition	-1.00000	-0.97911	-0.93314	-0.94300	-0.92640	1.00000

Growth Parameters: The growth parameters estimated were length of root and shoot, leaf count, the height of the plant, weight in terms of fresh and dry for root and shoot, root-shoot ratio, and moisture content. The results for all these parameters have been described graphically in Figures 3a, 3b, and 3c. The highest length of root (1.23 cm), shoot (4.7 cm), and plant height (5.90 cm) were observed at T_{20}. Several leaves were observed to be maximum (2.0) in T_{20} and T_{40} and declined further. All the growth parameters (root length-1.57 cm, shoot length-5.5 cm, leaf count-2.0, and height of the plant-7.07 cm) were recorded to be maximum with tap water.

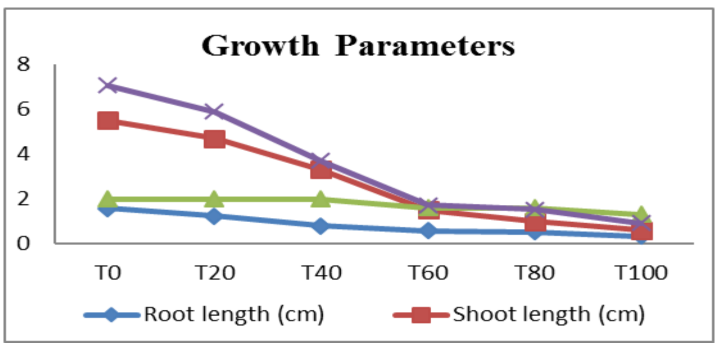

Figure 3a: Root and Shoot length at varied concentrations

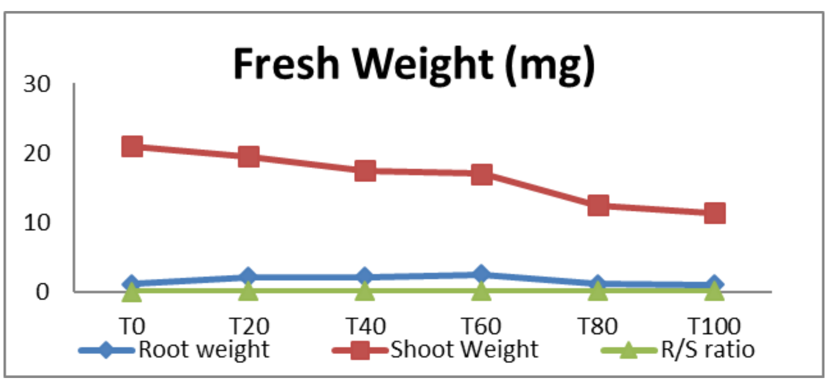

Figure 3b: Fresh weight of root, shoot and their ratios

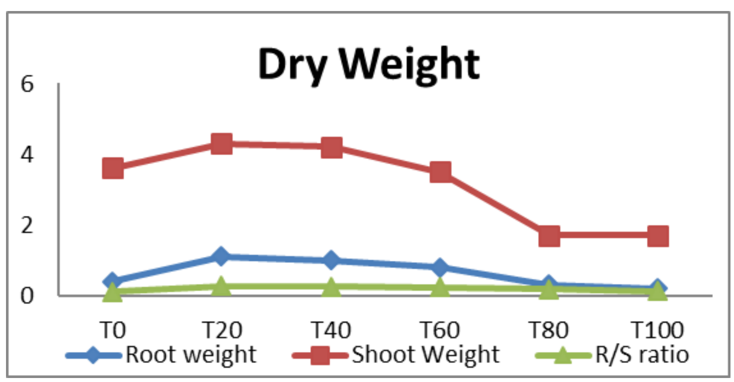

Figure 3c: Dry weight of root, shoot and their ratios

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Moisture content and Biomass: Evident from results, the fresh and dry weight improved with high application of wastewater at the initial stages of sowing season up to 60th day after sowing (DAS) as the maximum fresh weight of plant (28.42 g) and dry weight (6.07 g) was observed at T_{80} respectively. While beyondcertain days after sowing, it i.e., on 90th DAS, the weight began to decline. Maximum fresh weight (14.66 g) and dry weight (4.10 g) were recorded at T_{20}, respectively. At the same time, maximum (3.47 g) and minimum (0.26 g) values for dry weight stood at T_{20} and T_{100}, respectively, on 120th DAS. Tap water irrigation revealed fresh weight values as 11.12 g, 23.23 g, 14.42 g, and dry weight as 4.46 g, 4.10 g, and 3.75 g on 60th, 90th, and 120th DAS, respectively. On 60th DAS, moisture content was observed to be maximum (82.04%) at T_{40} and minimum (62.48%) at T_{100}. On 90th DAS, moisture content was found to be maximum (89.86%) at T_{100} and minimum (72.03%) at T_{20}. On 120th DAS, moisture content was recorded to be maximum (87.08%) at T_{80} and minimum (75.35%) at T_{20}.

Effect on Flowering: The effect of untreated paint industry wastewater at various concentrations on flower count, flower diameter, and fresh weight for flowers was analyzed every week. The number of flowers varied with the wastewater concentration. A maximum number of flowers (2.0), diameter (7.900 cm), and fresh weight (3.206 g) were observed at T_{20} in the first week of flowering whereas, a minimum number of flowers (1.0), diameter (6.200 cm), and fresh weight (0.967 g) were observed at T_{100}. In the second week of the maximum flowering number of flowers (4.0), diameter (8.100 cm), and fresh weight (4.202 g) were found at T_{40} and T_{20}, respectively. Whereas the minimum number of flowers (3.0), diameter (2.800 cm), and fresh weight (2.208 g) were observed at T_{60}, T_{20}, and T_{80}, respectively. A maximum number of flowers (6.0), diameter (5.500 cm), and fresh weight (5.555 g) were noticed at T_{40}, a minimum number of flowers (3.0), diameter (3.800 cm), and fresh weight (1.287 g) were noticed at T_{100} in the third week of flowering. It was also recorded, the weight of seeds declined as the application of wastewater was raised. The maximum weight of seeds (6234.5 mg) was observed at T_{20} and the minimum (2459.8 mg) at T_{100}. Tap water irrigation (T_0) showed the maximum weight (6776.1 mg) of seeds. ANOVA was performed to determine the difference in length of root and shoot and leaf count on the 60th, 90th, and 120th DAS. The difference was found out to be significant at a 5% level of significance (Table 1).

Table 1: ANOVA table for length of root and shoot as well as leaf count at different DAS

ANOVA

Source of Variation	SS	Df	MS	F	P-value	F crit
Root Length	44.467777 78	2	22.2338888 9	4.275565147	0.0339309 7	3.6823203 44
Shoot length	445.35444 44	2	222.677222 2	11.43955134	0.000961	3.68232
Number of leaves	21547.11	2	10773.56	17.75503	0.000111	3.68232

However, to see the impact of wastewater on length of root and shoot as well as leaf count, ANOVA was again performed and the results have been recorded below in Table 2. The above table revealed that difference amongst the treatments (T_0, T_{20}, T_{40}, T_{60}, T_{80}, T_{100}) measured across 60th DAS, 90th DAS and 120th DAS were not significant in terms of length of root and shoot as well as leaf count at 5% level of significance.

Table 2: ANOVA table for length of root and shoot as well as leaf count at different concentration level

ANOVA

Source of Variation	SS	Df	MS	F	P-value	F crit
Root Length	51.684444 44	5	10.3368888 9	1.752345074	0.1973817 16	3.10587523 9
Shoot Length	90.577777	5	18.1155555	0.336116437	0.8814125	3.10587523

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	78		6		18	9
Number of leaves	2383.6111 11	5	476.722222 2	0.202391622	0.9552564 91	3.10587523 9

To check the difference if fresh weight, dry weight and moisture measured on 60th DAS, 90th DAS and 120th DAS were significantly different, ANOVA has been used.

Table 3: ANOVA table for Fresh Weight, Dry Weight and Moisture at different DAS

ANOVA

Source of Variation	SS	Df	MS	F	P-value	F crit
Fresh Weight	70.99574	2	35.49787	0.767628	0.481507	3.68232
Dry Weight	12.68268	2	6.341339	2.754568	0.095737	3.68232
Moisture	404.55	2	202.275	3.675572	0.050227	3.68232

The above table showed that fresh weight, dry weight and moisture content measured on 60th, 90th and 120th DAS were significantly different. However, to observe the effect of wastewater on dry and fresh weight as well as moisture, ANOVA was again performed and the results are shown in below table.

Table 4: ANOVA table Fresh Weight, Dry Weight and Moisture at different concentration level

ANOVA

Source of Variation	SS	Df	MS	F	P-value	F crit
Fresh Weight	265.8928	5	53.17856	1.279468	0.33463	3.105875
Dry Weight	17.55184	5	3.510369	1.420119	0.285593	3.105875
Moisture	197.109	5	39.4218	0.457983	0.799995	3.105875

The above table showed that difference amongst the treatments (T_0, T_{20}, T_{40}, T_{60}, T_{80}, T_{100}) measured across 60th DAS, 90th DAS and 120th DAS were not significant in terms of fresh weight, dry weight and moisture at 5% level of significance.

Effect on Yield: To observe the effect on the yield of the test plant, the economic yield, stover index, and harvest index were calculated with different wastewater concentrations. The test plant's economic yield was maximum (10.325 g plant-1) at T_{60} and minimum (4.491 g plant-1) at T_{100}, which implied an increase in the economic yield using wastewater up to 60% but further higher up the yield declined. The stover yield was found to be highest (32.497) at T_{20} and lowest (6.075) at T_{100}, indicating the intolerance of paint industrial wastewater even at minimum concentration. The harvest index was found to be maximum (0.425) at T_{100} and minimum (0.170) at T_{20}, giving the inference that harvest index increased with the help of nutrients available in the paint industry wastewater. The economic yield decreased with the increase in wastewater application. The economic yield remained highest with tap water whereas, stover yield increased at 20 percent and further declined with the increase in wastewater concentration. Harvest index, on the other hand, was observed to be highest at 100% effluent concentration**(Fig. 4).**

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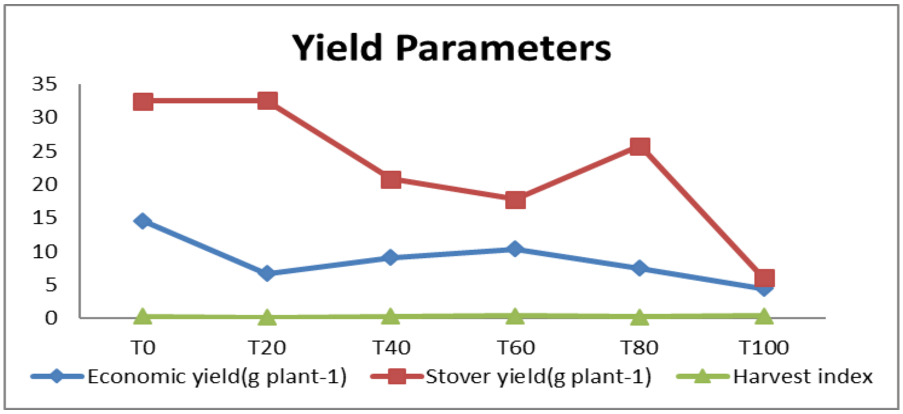

Figure 4: Various yield parameters with tap water and waste water at different concentrations

Pigment analysis: The pigments estimated using wastewater were chlorophyll a,b and total chlorophyll, as well as carotenoid content at various time intervals **(Fig. 5).**

Chlorophyll: Chlorophyll a was found to be highest (0.211 mg/g, 0.251 mg/g and 0.140 mg/g) at T_{20} whereas, least values (0.156 mg/g, 0.122 mg/g and 0.114 mg/g) were observed at T_{100} on 60th, 90th and 120th DAS respectively. Chlorophyll b was found to be maximum (0.055 mg/g, 0.096 mg/g and 0.048 mg/g) at T_{20} on 60th, 90th and 120th DAS and minimum values of chlorophyll b (0.045 mg/g, 0.044 mg/g and 0.034 mg/g) at T_{100} on 60th, 90th and 120th DAS. Tap water (T_0) revealed the values of chlorophyll b as 0.061 mg g-1, 0.059 mg g-1, and 0.033 mg g-1 on 60th, 90th, and 120th DAS, respectively. With a unique difference, it was found out that the highest value of total chlorophyll (0.301 mg/g) was observed at T_{100} on 60th DAS. Whereas on 90th and 120th DAS, the maximum was (0.347 mg/g and 0.180 mg/g) values were observed at T_{20}. T_0 revealed the total chlorophyll as 0.272 mg/g, 0.222 mg/g, and 0.131 mg/g on 60th, 90th, and 120th DAS. Thus, it was found that chlorophyll a and b increased at lower concentrations but decreased at higher concentrations. It was also found that the chlorophyll a and b declined with time and raised the application of wastewater whereas, total chlorophyll showed a varied trend over the period.

Carotenoids: The maximum value of carotenoids was observed as 0.104 mg/g and 0.115 mg/g at T_{20} on 60th and 90th DAS, respectively, and 0.056 mg/g at T60 on 120th DAS. Whereas minimum values (0.093 mg/g, 0.050 mg/g, and 0.043 mg/g) were observed at T_{100} on 60th, 90th, and 120th DAS, respectively. Tap water irrigation revealed the values to be 0.103 mg/g, 0.070 mg/g, and 0.038 mg/g on 60th, 90th, and 120th DAS. Thus, it was recorded that there was a substantial rise in the pigment content at lower wastewater applications whereas, it declined at raised levels of applications.

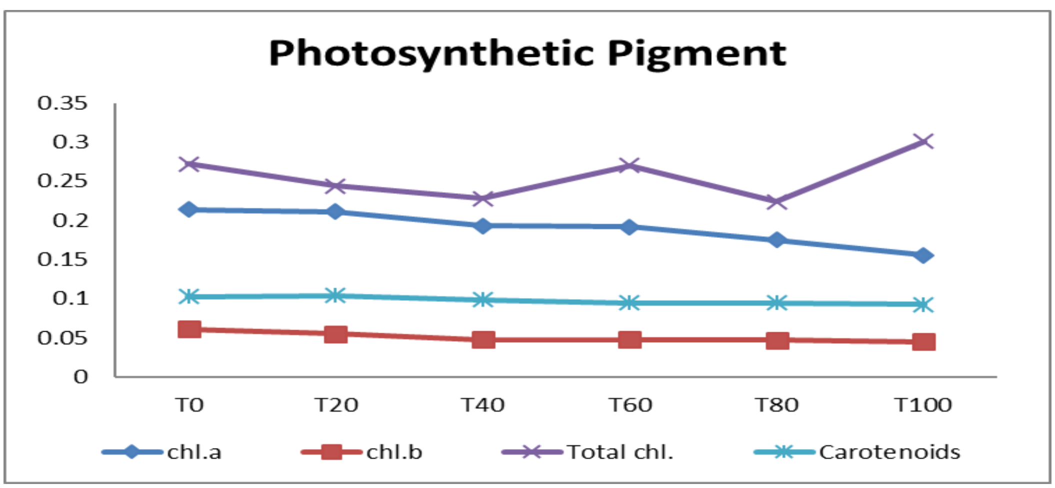

Figure 5: Pigment determination (Chl a, b and total chlorophyll and carotenoids) at varied concentrations

Discussion

Several studies have been conducted so far in this field, where various industrial wastewaters are used to check the irrigation potential of various crops. The experiments have been conducted with treated as well as untreated wastewater. Paint industrial wastewater studies conducted by various workers are Aniyikaiye et al., 2019; Damaraju et al., 2019; Bhat et al., 2018; Chidozie and Nwakanma, 2017; Guven et al., 2017; Krihika and Ligy, 2016; Olaoye and Oladeji, 2015; Jolly et al., 2008. Various studies by a variety of researchers carried out using a range of industrial wastewater on different crops are by Lallawmsanga et al., 2012; Vijayaragvan et al., 2011; Malaviya and Sharma 2011; Kumar, 2011; Yousaf et al., 2010; wins and Murugan (2010); Ogunwenmo et al., 2010; Singh, 2010; Nagajyothi et al., 2009; Rehman et al., 2009. Our findings were quite close to the studies conducted by Patro et al., 2007; Malaviya et al. (2007); Garg and Kaushik, (2007); Nath et al. (2007); Singh et al.(2006); Pandey (2006); Pragasam et al. (2005), Singh et al. (2004). Pieces of evidence were also presented by Gouia et al.(2003); Singh et al. (2002); Ramana et al. (2002); Augusthy and Mani, (2001); Reddy and Borse, (2001); Javaid et al.(2000); Prasanna et al. (1997), Leubner-Mergzer et al.(1995), Krupa et al.(1993), Kelly et al.(1992), Groot et al.(1992) Gautam and Bishnoi (1990), Choudhury and Choudhury(2014) Purkayastha, P et al. (2015). Our study was different because the untreated paint industry wastewater did not support the plant growth and development even at the lowest percentage of 20% concentration and 80% water.

Conclusion

The paint industry wastewater proved to be toxic for *Tagetes erecta*, as revealed through results and statistical analysis performed on them. The germination parameters showed that with increasing concentration, the positive germination parameters showed a decline while the negative parameters increased. Germination is the positively affected parameter of *Tagetes erecta* with untreated wastewater from the paint industry at lower concentrations but not at higher dose. The case of other parameters like growth, yield, and pigment contents revealed a rising trend at initial applications upto 60^{th} DAS but declined further with higher dose and number of DAS. Economic output enhanced with the rise in the wastewater up to a certain level, whereas it declined further. The harvest index showed to be highest at the highest concentration of 100%. Therefore, paint industry wastewater requires pre-treatment before it is discharged into the environment or released into drain drains from where it is further carried to the agricultural fields. The correlation table showed the relationship between the parameters. The closely related parameters were expected to have a high correlation and vice-versa.

Acknowledgements

We, as authors, are thankful to the Department of Environmental Sciences, University of Jammu for providing the support for experimenting. The authors are also thankful to Berger Paints Limited for permitting to collect the wastewater. Help rendered by all colleagues and friends is deeply acknowledged.

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