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ORIGINAL ARTICLE

Effects of Fertilization with Textile Effluent on Germination, Growth and Metabolites of Chilli (*Capsicum annum* L) Cultivars

Ratan Singh[1] · Dheeraj Rathore[1]

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Abstract
Nutrient deficiency in soil suppresses crop growth, yield and nutritional value of the products. Textile effluent, a rich source of several essential minerals (including Ca, Mg, Cu, Fe, Zn, Mn) required for plant growth, could be a viable option to supplement the nutrient availability of soil. Although presence of some toxic metals and organic compounds restrict its use as irrigation water, its controlled use as fertilizer was not studied so far. This study was undertaken to assess the utilization of textile industry effluent for its suitability and potentiality as nutrient supplement by applying it onto chilli (*Capsicum annum* L.) cropping system. The effects of textile effluent fertilization was assessed by the wet cotton method at room temperature for germination, and in pot experiment in field conditions for growth and biochemical analysis using four effluent dilution rates (i.e., 10%, 20%, 40% and 60% v/v). The results of the experiment showed no inhibitory effect of textile effluent on seed germination, while its fertilization as soil drench worked as nutrient supplement for growth in chilli cultivars. The fertilization with textile effluent enhanced the plant biomass by 110.9% and 124.5%, and the leaf area by 21.5% and 2.6% in chilli cultivar GVC-121 and GVC-101, respectively. The total carbohydrate and foliar protein were also favoured by fertilization with the effluent. Moreover, least proline accumulation under lower dose suggested reduced stress due to textile effluent fertilization. The study concluded that the lower dose of textile effluent fertilization can function as nutrient supplement for chilli cultivars and 20% (v/v) effluent dilution provides the most favourable results.

Keywords Textile effluent · Fertilization · *Capsicum annum* · Germination index · Growth · Metabolites

Highlights
- The application of textile effluent enhances germination of chilli cultivars.
- A low dose of textile effluent provides nutrients and increases growth.
- High dose of textile effluent delays germination and reduces growth.
- Application of wastewater to agricultural field may be a viable management option.

1 Introduction

Effluent of the textile industry is considered to be among the most polluted industrial effluents consisting of a high concentration of heavy metals and organic compounds (Singh et al. 2020; Singh and Rathore 2020). Besides, the textile industry is also known as a water intensive industry, consuming large quantities of water for various processes and discharging equally large volumes of wastewater containing a variety of pollutants (Kumar and Chopra 2013; Bhatia et al. 2018; Singh and Rathore 2019). The textile effluent is also characterized by the presence of organic dyes that develop strong colour, fluctuating pH, high chemical and biological oxygen demands, total dissolved solids, sulphate, and ammonical nitrogen (Kaushik et al. 2005; Grönwall and Jonsson 2017) which further complicate the problem of its management. The unimpeded disposal of textile effluent not only causes risks to human, animal and plant health but also possesses serious threats to soils and water bodies, and ecology of affected areas (Saratale et al. 2011; Forss et al. 2017; Singh et al. 2020). Fait et al. (2017) suggested that the uncontrolled disposal of waste could lead to distribution of metallic trace elements into the soil from top of the soil to deep up to one km through the soil-water interface. Textile effluent can induce mutations, and cause genotoxicity and oxidative damage, root growth retardation, mito-depression, and induction of chromosomal abnormalities in root meristematic cells (Hemachandra and Pathiratne 2016; Akhtar et al. 2016). Iqbal (2016), Abbas et al. (2018) and Iqbal et al. (2019) reviewed the available literature and concluded cytogenic and mutagenic effects of raw textile effluent on *Vicia faba*, *Vibrio Fischeri* and *Allium cepa*, respectivey, and suggested biological treatment of the effluent before its use.

On the other hand, micronutrient deficiency in soil and plants is a global nutritional problem and is prevalent in many countries with different magnitude of severity. Micronutrient deficiencies in soil have been identified as one of the main factors affecting crop yield and food quality, which resulted in reduced nutrient in diet (Cakmak 2002; Singh et al. 2018). Widespread micronutrient deficiency increases the challenge to feed healthy food for 7 billion people which is expected to reach 9 billion by 2050 (FAO 2011). Therefore, sufficient key nutrients are required in soil for successful agricultural practice (Knez and Graham 2013). The industrial effluent contains several macro and micronutrients which the plants can uptake for their growth. Application of such effluent in agricultural fields may be a viable option to dispose industrial effluent, and would sustain agriculture in non-irrigated areas where the availability of fresh water is scarce (Kumar and Chopra 2013). Moreover, waste water can provide important nutrients, especially nitrogen and phosphorus and some micro-nutrients, which can increase soil fertility and enhance plant growth, crop production and quality of produce. It also reduces the requirements for commercial chemical fertilizers, and thus, it increases farmer's economic benefits (Papadopoulos and Savvides 2003). Fertilization by effluent may provide dual benefit to the environment as it reduces the requirement of chemical fertilizers besides resolving the problem of effluent disposal (Knez and Graham 2013; Kumar and Chopra 2013). Tran et al. (2016) also discussed his experimental result that the reuse of wastewater in agriculture offers cost savings over chemical fertilizers as it contains considerable amount of nutrient elements for plants.

Besides the presence of toxic heavy metals and organic compounds, metals necessary for plant metabolism as enzyme activators or regulators are present in textile effluent, e.g. Fe, Cu, Mn, Mo; however, these may cause toxicity if supplied in excess quantities (Kaushik et al. 2005; Singh and Rathore 2018). Use of wastewater reduces fertilizer and irrigation cost as it is freely available (Papadopoulos and Savvides 2003). Earlier researches with lower dose of distillery effluent as irrigation provided positive results on seed germination, total sugars, starch, reducing sugars, and chlorophyll (Ramana et al. 2002; Kaushik et al. 2005; Hassan et al. 2013; Singh and Rathore 2018, 2020).

The studies conducted so far on the utilization of textile effluent in agriculture were focused on nutrient utilization during irrigation. However, the presence of high salinity and significant amounts of trace metals can harm the crop and soil by its continuous application as irrigation. Djehaf et al. (2017) reviewed the available treatment technologies for textile effluent and suggested its use for field application after treatment. Use of diluted treated wastewater could somewhat mitigate the side effects without additional treatment; it is considered that diluted wastewater can be used for soil fertilization (Bañón et al. 2011; Jeong et al. 2016). Singh and Rathore (2020) suggested to use diluted textile effluent during the field preparation to satisfy the need of micronutrients to plants. This would not only reduce the input of chemical fertilizer but also reduce the cost of textile effluent disposal.

Chilli (*Capsicum annum* L.) is an important commercial spice crop which is a rich source of vitamin 'C' and 'A' with plenty of minerals (Parvathi and Yurnus 2010). Global trade of chilli is around 400,000 metric tons. Alabi (2006) stated that chilli shows greater yield and quality when essential elements are provided in appropriate amounts. So far, no study has been conducted on the application of limited amount of textile effluent as fertilizer to provide nutrients from textile effluent on chilli crop. Therefore, the present study was conducted to assess the potential of textile industry effluent to supplement micronutrients during cultivation of chilli (*Capsicum annum* L. cv.GVC-101 and GVC-121) by fertilization with different concentration of effluent, and to identify the optimum effluent concentration for fertilization of chilli cultivars.

2 Materials and Methods

2.1 Effluent Collection and Characterization

Textile effluent originating from the Mangalam textile industry was collected in well cleaned unused plastic containers from a Green Environment common effluent treatment plants (CETP) located in Vatwa, Ahmedabad, India (Singh and Rathore 2020) before the treatment process. The collected samples were stored in refrigerator (4 °C) to avoid changes in their characteristics. As the textile effluent used is the present study was same to that of our previous studies (Singh and Rathore 2018, 2020), its physico-chemical characteristics, determined using Standard Methods (APHA 2012), were also similar (Table 1) as reported in Singh and Rathore (2018, 2020).

2.2 Germination Experiment

The wet cotton method was applied for the germination experiment. Cotton was moistened with 10 mL of water as control and with the same quantity of different concentrations of textile effluent resulting from 10%, 20%, 40% and 60% *v*/v dilution in water and kept in a Petri dish. Eight seeds of the two cultivars were placed in these Petri-dishes and were incubated at temperature of 30 ± 1 °C. Germination was recorded every 5 days from the date of sowing for 15 days at 11:00 am, and the emergence of the radicle was taken as a criterion of germination. All the experiments were carried out in three replicates (3 Petri dishes for each treatment) and the results were averaged.

Table 1 Physico-chemical Characteristics of textile effluent (obtained from Singh and Rathore 2018)

Physical Properties	
Temperature	38 °C
Colour	Brownish
Odour	Fishy
pH	6.86±0.1
Chemical properties	
Alkalinity (mEq L^{-1})	643±0.21
EC (μS cm^{-1})	438±0.43
TS (mg L^{-1})	2043±1.94
TSS (mg L^{-1})	97±1.63
TDS (mg L^{-1})	1909±1.08
COD (mg L^{-1})	1587±1.34
chloride (mg L^{-1})	693±0.51
sulphate (mg L^{-1})	286±0.37
Phosphate (mg L^{-1})	1.58±1.08
Phenols (mg L^{-1})	173.05±0.07
Total nitrogen (mg L^{-1})	80.13±1.5
Ca (mg L^{-1})	60.63±0.01
Mg (mg L^{-1})	ND
Elemental Properties	
Fe (mg L^{-1})	1.2±0.063
Zn (mg L^{-1})	2.16±2.26
Mn (mg L^{-1})	1.1±0.05
K (mg L^{-1})	23.44±0.87
Cr (mg L^{-1})	4.22±2.34
Pb (mg L^{-1})	ND
Cd (mg L^{-1})	1.3±0.004
Co (mg L^{-1})	0.9±0.01
Cu (mg L^{-1})	1.3±0.002
Al (mg L^{-1})	14.3±0.03
Ni (mg L^{-1})	1.05±0.33
Biological properties	
BOD_5 (at 20 °C) (mg L^{-1})	688±1.08
CFU (mL^{-1} × 10^3)	1.02× 10^6
MPN/100 mL	32.46

Seed germination for the various effluent concentrations was recorded and computed as germination percent. Speed of germination was analysed using the Eq. (1):

$$\text{Speed of germination} = \frac{\text{No. of seeds germinated}}{\text{No. of days from the first count}} + \ldots + \ldots + \frac{\text{No. of seeds germinated}}{\text{No. of days of the final count}} \quad (1)$$

Peak value and germination value were calculated using Eqs. (2) and (3) presented by Kaushik et al. (2005):

$$\text{Peak value} = \frac{\text{Cumulative percent germination on each day}}{\text{No. of days elapsed since initial imbibition}}. \quad (2)$$

$$\text{Germination value} = \text{Peak Value} \times \text{germination percent}. \quad (3)$$

Seed Vigor index and delay index were obtained using the following Eqs. (4) and (5) explained in Abdul-Baki and Anderson (1973):

$$\text{Vigor index} = \text{Germination percent} \times \text{mean of seedling length (root + shoot) at the end of incubation period.} \quad (4)$$

$$\text{Delay index} = \frac{\text{Delay in germination time over control}}{\text{Germination time for control}}. \quad (5)$$

2.3 Pot Experiment Material and Design

The pot experiment was conducted at the nursery of Gujarat Forest Research Foundation (GFRF), Gandhinagar, India (23.2362° N, 72.6766° E). Seeds of chilli (*Capsicum annum*) cultivars, i.e., GVC-101 and GVC-121 were obtained from Anand Agricultural University, Anand, Gujarat. Both cultivars are grown in winter-monsoon season, but are different in their morphology, biochemical characteristics, nutritional composition and yield potential (https://www.aau.in; Litoriya et al. 2014). Genetically, uniform seeds of chilli were sown in pots of 19 cm (diameter at top) × 18 cm (height) size. Pots were filled with equal amounts of slightly alkaline (pH 7.8, EC 0.53 μScm^{-1}) sandy loam soil of medium fertility (N, P, K values of used soil were 236.31, 78.63, 118.54 kg ha^{-1}, respectively). Trace element values of the experimental soil before addition of textile effluent were: Fe 60.6 ± 0.11; Zn 0.21 ± 0.01; Mn 1.28 ± 0.04; As 0.0028 ± 0.21; Cd 0.0 ± 0.0; Co ND; Al 92.8 ± 1.03, Ni 0.30 ± 0.01; Pb 0.2 ± 0.003 (all values are mg kg^{-1} of soil) (Singh and Rathore 2020). Twelve seeds of chilli cultivars were sown in each pot. Four dilution rates of textile effluent (10%, 20%, 40% and 60% *v*/v, i.e., T1, T2 T3 and T4, respectively) were applied in soil as the basal fertilizer dose for micronutrient supplementation (Table 2). Fertilization with textile effluent was applied only once during the soil preparation. Pots were fertilized by textile effluent before the sowing of seeds and left for a week for proper mixing. A control set (without textile effluent fertilization) was also maintained for comparison. Ground water was applied for irrigation purpose in all treatments. After germination seeds were thinned to six seedlings per pot in all the pots, which were further thinned at each sampling period. The experiment was conducted under completely randomized design and replicated three times. For the various physiological, biochemical and elemental analyses, the chemicals were purchased from Sigma-Aldrich, Bangalore, India. Other acids, buffer components, major and minor salts were obtained from MERCK and Himedia Mumbai, India. All chemicals were purchased in highest purity and either of analytical or HPLC grade as required.

2.4 Plant Growth Analysis

Plants were randomly sampled in triplicate from all treatments at 30, 60, 90 and 120 days after sowing (DAS). After carefully washing with distilled water, plants were separated into roots, stem and leaves. Plant height (roots and shoot length) was measured by meter scale and leaf area was measured by graphical method and presented as cm^2 per plant. To determine biomass, plant parts were oven-dried at 80 °C till a constant weight was achieved. After drying, the plant

Table 2 Volume of textile effluent used

Treatment	Quantity of textile effluent (mL textile effluent/kg soil)
C	0
T1	110
T2	220
T3	440
T4	660

parts were weighed by weighing balance. Root to shoot ratio (RSR) was calculated according to Hunt and Burnett (1973).

2.5 Photosynthetic Pigments

For chlorophyll (Chl) and carotenoid determinations, 0.1 g leaf sample was placed in 10 mL of 80% acetone in a test tube and kept it overnight in a refrigerator at 4 °C. It was then homogenized and centrifuged at 6000×g for 15 min. The optical densities of the supernatant were measured at 480, 510, 645 and 663 nm using a spectrophotometer (Systronic, Model 2203). The contents of Chl *a*, Chl *b* and carotenoid were calculated as explained in Maclachlan and Zalik (1963) and Duxbury and Yentsch (1956), respectively. Total chlorophyll was obtained by adding the value of Chl *a* and Chl *b*.

2.6 Metabolites and Proline Content

For carbohydrate, 1 g of fresh leaf sample was crushed with chilled 70% ethanol and centrifuged at 4000 rpm for 10 min. After centrifugation, 1 mL of obtained supernatant was added with freshly prepared 4 mL of anthrone reagent and the mixture was allowed to stand in warm water for 8–10 min, then cooled rapidly and absorbance was read to estimate total carbohydrate content as described by Yemm and Willis (1954).

Protein was estimated by leaf extraction in 0.2 M phosphate buffer of pH 7 following the method of Lowry et al. (1951). Amount of protein present in the samples was expressed with the bovine serum albumin (BSA) as standard in $\mu g\ mL^{-1}$.

Proline content was estimated by extracting the leaf sample in 10 mL sulfosalicylic acids and the supernatant was used for acid-ninhydrin test (Bates et al. 1973) and expressed as μmol proline g^{-1} FW.

2.7 Statistical Analysis

The data for different treatments are presented as mean value of three replicates. and standard errors. The significance of the data was analysed using two ways ANOVA, with growth stage and treatments considered as two factors. Statistical analyses were performed using the SPSS program (version 17.0) to compare the effect of textile effluent fertilization and plant age. Plant photosynthetic pigment content, biochemical characterisation, and biomass assay were compared by analysis of variance and multiple comparison tests. In case of significant changes, heterogeneous groups were distinguished on the basis of duncan multiple range test (DMRT) at $p < 0.05$.

3 Results

3.1 Effluent Characteristics

The used textile effluent was brownish in colour, deficient in dissolved oxygen, rich in total solids, total alkalinity, high in biological oxygen demand (BOD) and chemical oxygen demand (COD) and with considerable amounts of total nitrogen, phosphate, chlorides, sulphate, sodium, calcium, zinc, manganese, copper, nickel, ferrous, lead and cadmium (Singh and Rathore 2018). The concentration of total suspended solids (TSS), BOD, COD, and total dissolved solids (TDS) of effluent used in the study exceeded the prescribed limits of Indian irrigation standards (BIS 1991). Bhatia et al. (2018) also reported increased pollutant indices of textile effluent; however, nutrient content was not analysed.

3.2 Germination Experiment

Textile effluent dilution favoured well both chilli cultivars in the germination experiment. No inhibition on germination by textile effluent was seen on the seeds. Germination percent (GP) of both chilli cultivars was significantly higher after fertilization with textile effluent than control plants (Table 3). Maximum increase in germination percent was seen for the lowest dose of textile effluent (T1), and GP decreased gradually with increasing concentration. Moreover, significantly higher speed of germination (SOG) and peak value (PV) were also observed for textile effluent fertilization with experimental chilli cultivars except for the highest dose of textile effluent fertilization which showed slightly lower SOG than the control. Similarly, germination value (GV) and seed vigor index (SVI) were also higher for textile effluent fertilization with maximum increase under lower dose of textile effluent. However, the delay index (DI) was higher under the higher dose of textile effluent. In general, germination indices of cultivar GVC-101 were more responsive to textile effluent fertilization.

3.3 Plant Growth

Application of textile effluent showed positive response for shoot height and root length in both chilli cultivars (Fig. 1a) at all ages. For shoot height, most suited fertilization dose of textile effluent was T2 at all ages except 30 DAS in GVC-101 which showed maximum growth for T1. Highest shoot lengths of cultivars GVC-101 and GVC-121 were observed at 31.17% and 39.25%, respectively, at 120 DAS for T2 treatment, while root length was highest under T3 treatment at 120 DAS (31.56% and 18.91% for GVC-101 and GVC-121, respectively). Factorial analyses showed significant variation for all factors in both cultivars except interaction of age and treatment for cultivar GVC-101 for root length (Table 4).

Similar to plant height, leaf area was also increased by textile effluent fertilization in both studied cultivars and best result was seen for T2 treatment (Fig. 1b). Increase in leaf area was significant by both factors, i.e., plant age and fertilization dose and its interaction for both experimental cultivars (Table 4). Highest leaf areas, i.e., 26.59 and 21.46 cm^2 $plant^{-1}$ for cv. GVC-101 and GVC-121, respectively, were found at 60 DAS for T2 treatment.

Plant biomass was significantly increased at successive growth stages under the lower dose of textile effluent fertilization for both experimental cultivars (Fig. 1c). However, the higher dose of textile effluent (T4) had adverse effect on biomass accumulation for the tested chilli cultivars. Application of textile effluent fertilization with 20% (T2) dilution was found most

Table 3 Effect of textile effluent on Germination percent (GP), Speed of germination (SOG), peak value (PV), germination value (GV), seed vigor index (SVI) and delay index (DI) of chilli (*Capsicum annum*) cultivars GVC-101 and GVC-121. Rows with different letters indicate statistically significant differences between treatments according to Duncan's multiple range test (at $p < 0.05$)

Parameter	GVC-101 Treatment					GVC-121 Treatment				
	C	T1	T2	T3	T4	C	T1	T2	T3	T4
GP	56.67 ± 0.04^{a}	85.78 ± 1.03^{b}	77.89 ± 0.11^{ab}	74.11 ± 0.05^{ab}	62.00 ± 0.04^{ab}	68.33 ± 1.33^{a}	88.00 ± 0.01^{a}	82.22 ± 0.15^{a}	69.00 ± 0.02^{a}	65.11 ± 0.17^{a}
SOG	21.81 ± 1.42^{b}	30.35 ± 1.09^{e}	26.73 ± 0.01^{d}	25.27 ± 0.08^{c}	21.17 ± 0.03^{a}	22.21 ± 1.27^{b}	31.33 ± 0.31^{c}	28.54 ± 0.33^{bc}	24.84 ± 1.24^{ab}	22.29 ± 0.42^{a}
PV	11.33 ± 0.07^{a}	17.16 ± 0.01^{e}	15.58 ± 0.06^{d}	14.82 ± 0.03^{c}	12.40 ± 0.04^{b}	13.67 ± 0.01^{a}	17.60 ± 0.04^{b}	16.45 ± 0.11^{b}	13.80 ± 0.14^{a}	13.02 ± 0.08^{a}
GV	642.2±0.1^{a}	1471.6 ± 0.3^{e}	1212.7 ± 0.1^{d}	1098.6 ± 0.2^{c}	768.8 ± 0.1^{b}	933.9 ± 0.2^{b}	1548.8 ± 0.3^{d}	1352.1 ± 0.4^{c}	952.2 ± 0.2^{b}	847.90 ± 0.1^{a}
SVI	75.75±0.1^{a}	227.06 ± 0.2^{d}	218.88 ± 0.06^{d}	161.14 ± 0.11^{c}	109.81 ± 0.3^{b}	22.13 ± 0.23^{a}	209.44 ± 0.07^{d}	210.84 ± 0.13^{e}	150.22 ± 0.02^{c}	110.05 ± 0.14^{b}
DI	–	0 ± 0.00^{a}	0 ± 0.00^{a}	0.5 ± 0.002^{c}	1 ± 0.001^{b}	–	0 ± 0.00^{a}	0.2 ± 0.001^{ab}	0.5 ± 0.001^{c}	1.5 ± 0.001^{b}

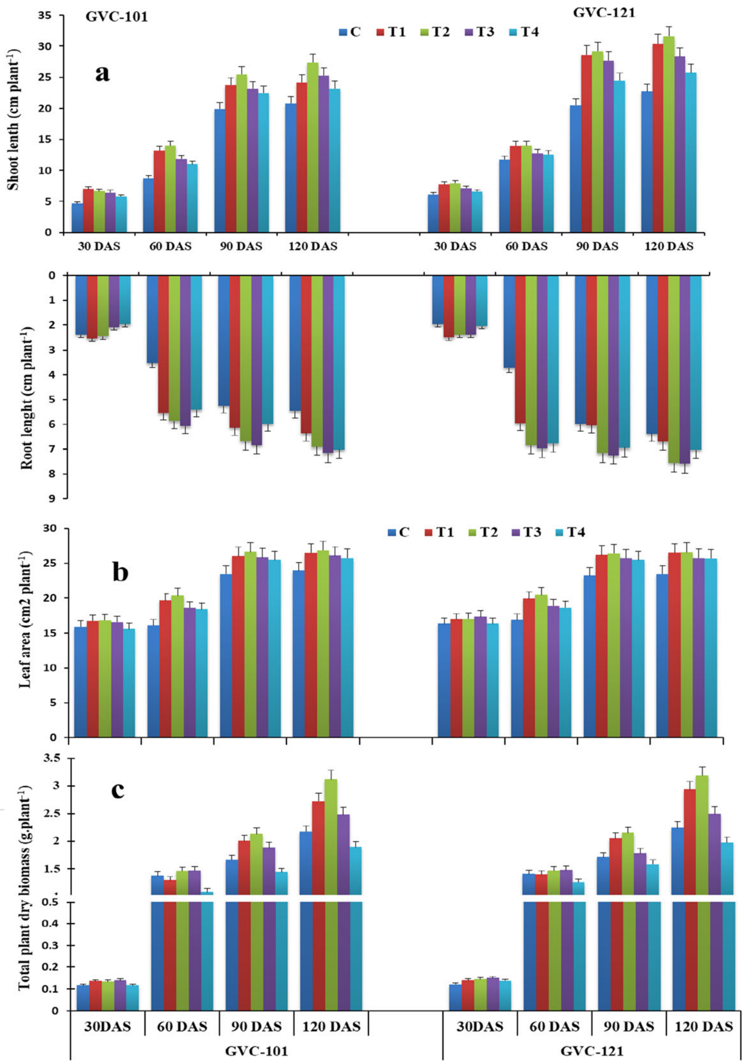

Fig. 1 (**a**) Shoot height and root length, (**b**) leaf area, and (**c**) total dry biomass of chilli (*Capsicum annum*) cultivars (GVC-101 and GVC-121) at 30, 60, 90 and 120 days after sowing (DAS) for the different treatments (different dilution rates of textile effluent fertilization)

suited for total biomass accumulation in both cultivars. Highest increase of total dry biomass was recorded with T2 treatment at 120 DAS for both chilli cultivars, i.e., 43.78% and 42.19% for GVC-101 and GVC-121, respectively, against their respective controls. However, factorial analysis for biomass showed non-significant effect of textile effluent on cultivar GV101 (Table 4).

3.4 Photosynthetic Pigments and Plant Metabolites

Chlorophyll *a* content accelerated significantly from 30 to 120 days in both cultivars at all textile effluent fertilization doses (Fig. 2a). Increase in Chl *a* was found maximum at 120

Table 4 Summary of two-way analysis of variance (ANOVA) at variable levels of significance (*p < 0.05, **$p < 0.01$, ***$p < 0.001$ and NS-not significant)

Parameter	Age		Treatment		Age×Treatment	
	GVC-101	GVC-121	GVC-101	GVC-121	GVC-101	GVC-121
Leaf area (LA)	*	***	**	***	**	***
Root length	***	***	***	***	NS$^{(0.127)}$	***
Shoot height	***	***	***	***	***	***
RSR	***	***	NS$^{(0.131)}$	**	NS$^{(0.415)}$	**
Chlorophyll -*a*	***	***	***	***	***	***
Chlorophyll -*b*	***	***	***	***	**	**
Total chlorophyll	***	***	***	***	***	**
Carotenoid	***	***	***	***	***	***
Protein	***	***	***	***	***	***
Total carbohydrate	***	***	***	***	***	***
Proline	***	***	***	***	NS$^{(0.425)}$	NS$^{(0.560)}$

DAS (45.25% and 32.66% for GVC101 and GVC-121, respectively) for T2 treatment compared to their respective controls. Similarly to Chl *a*, Chl *b* was also increased in a similar trend (Fig. 2b). Plant fertilized by 40% (T3 treatment) textile effluent exhibited maximum content of total chlorophyll (46.32 and 35.07%, respectively, for cultivar GVC-101 and GVC-121) than control at 120 DAS (Fig. 2c). Analysis of variance showed highly significant effect of both factors and their interactions for chlorophyll content of tested cultivars (Table 4).

Carotenoids content recorded an increase with successive plant age in both tested cultivars (Fig. 2d). Carotenoids content was not much affected by T1 treatment; however, it was found increased with increasing dose. Increase was observed maximum under T3 treatment for both tested cultivars. Highest carotenoids in both cultivars was found at 90 DAS with T3 treatment. Statistically, effect of age, treatment and their interaction was highly significant with both cultivars (Table 4).

Total carbohydrate content was affected positively by textile effluent fertilization in both chilli cultivars of the present experiment (Fig. 3a). Increase in carbohydrate was gradual up to T2 treatment then declined. Highest increase in total carbohydrate content was seen in T2 treatment at 90 DAS for GVC-101 (78%) and in T2 treatment at 120 DAS for GVC-121 (83%). Similar to total carbohydrate, protein content was also increased significantly under textile effluent application in a trend similar to carbohydrate at all ages with all treatments in both tested cultivars (Fig. 3b). Protein content was observed maximum in GVC-101 (72.92%) at T2 at 120 DAS. Order of the increase was T2 > T1 > T3 > T4 in both cultivars compared to their respective control. Variation in total carbohydrate and total protein was statistically significant for both factors viz. age and textile effluent fertilization and their interaction (Table 4).

Contrary to carbohydrate and protein, proline accumulation was reduced at successive ages by textile effluent fertilization in respect to their control except at 30 DAS, where it was found higher in T1 treatment (Fig. 3c). Proline accumulation was least with T2 fertilization in both cultivars. Analysis of variance showed highly significant effect of individual factors, although factor interaction was non-significant (Table 4).

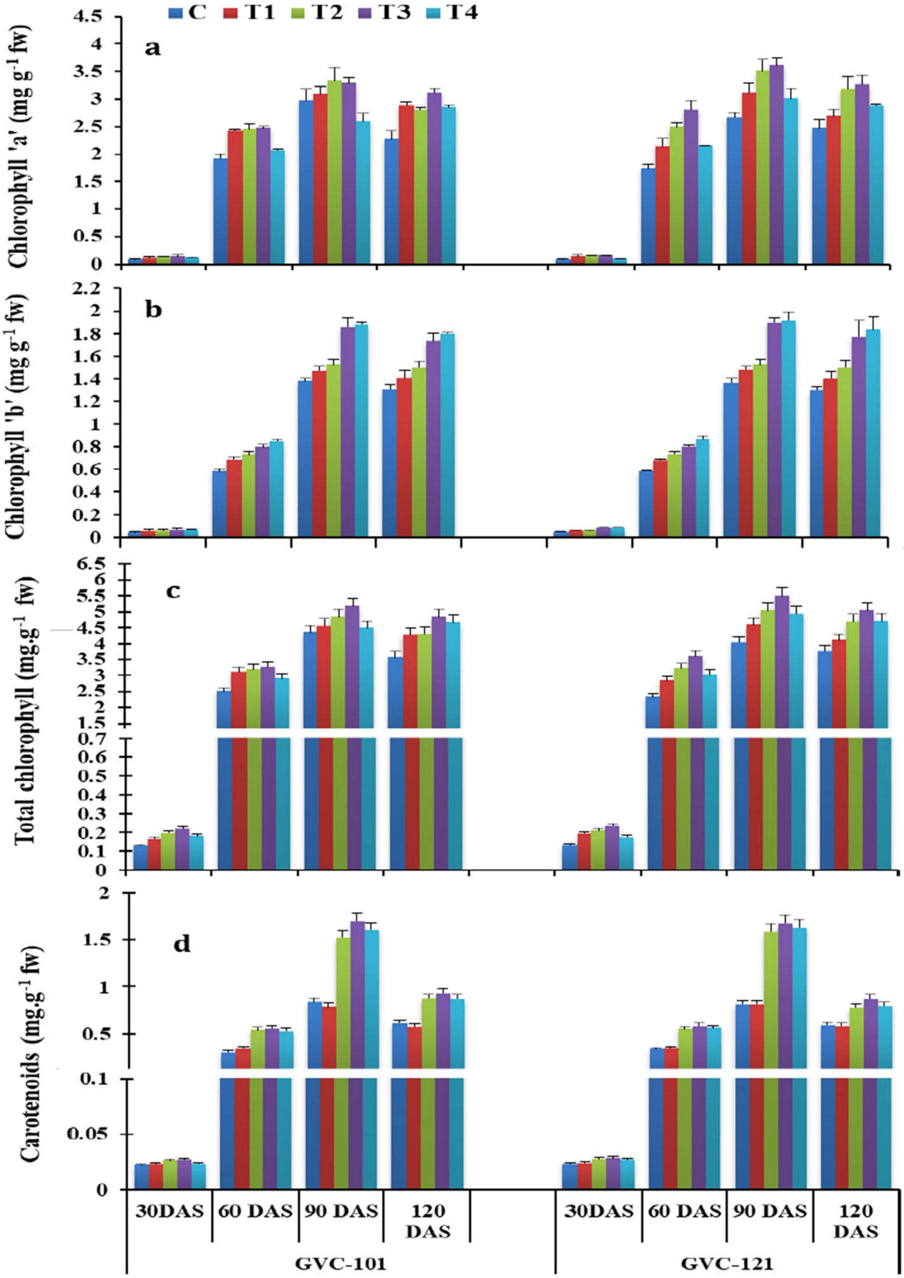

Fig. 2 (**a**) chlorophyll-*a*, (**b**) chlorophyll-*b*, (**c**) total chlorophyll, and (**d**) carotenoids content in leaves of chilli (*Capsicum annum*) cultivars (GVC-101 and GVC-121) at 30, 60, 90 and 120 days after sowing (DAS) for the different treatments (different dilution rates of textile effluent fertilization)

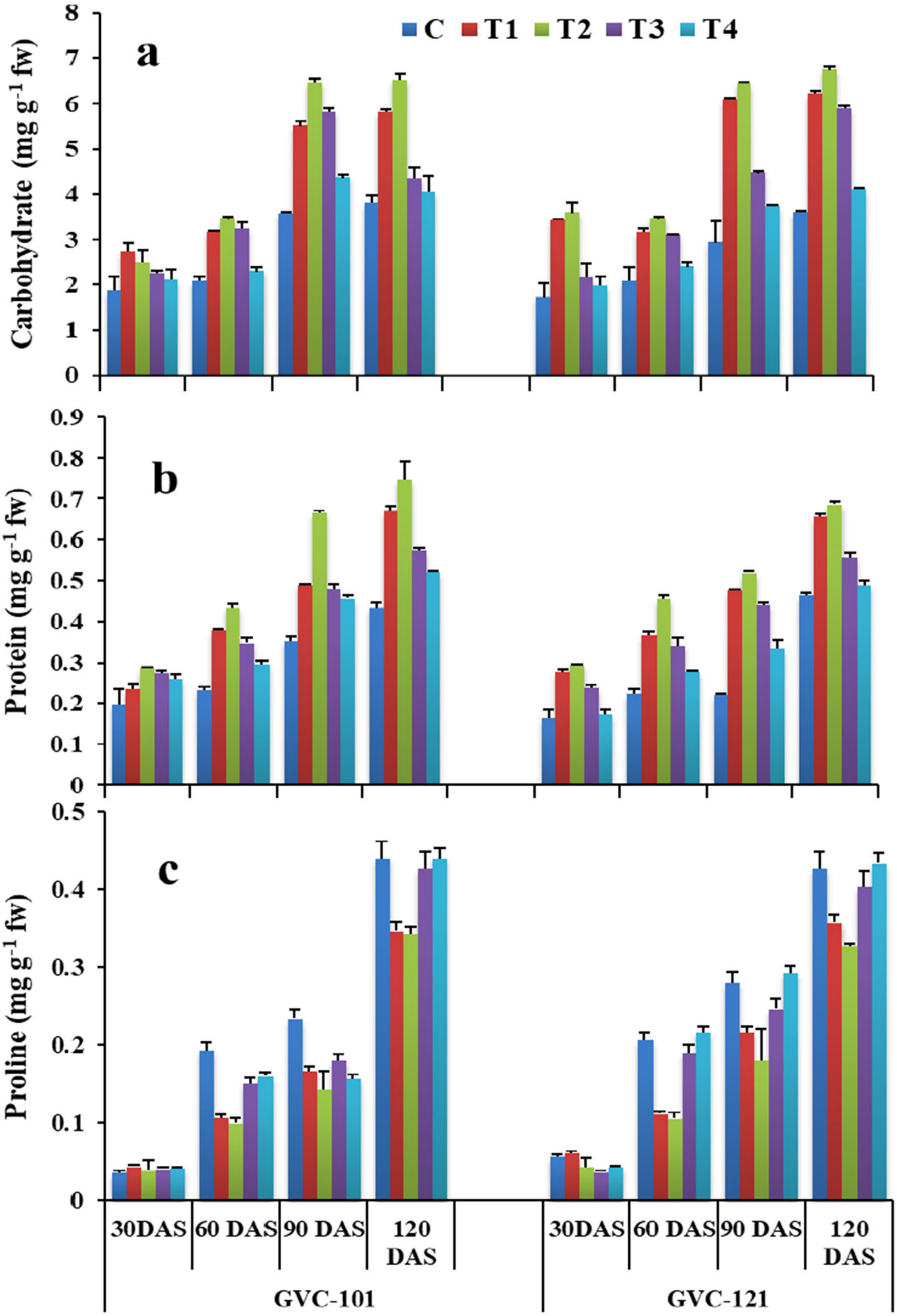

Fig. 3 (**a**) Carbohydrate content, (**b**) protein content, and (**c**) proline accumulation in leaves of chilli (*Capsicum annum*) cultivars (GVC-101 and GVC-121) at 30, 60, 90 and 120 days after sowing (DAS) for the different treatments (different dilution rates of textile effluent fertilization)

4 Discussion

Textile effluent plays a major role in producing large amounts of water pollution by characteristic toxicity of its effluent, i.e., higher total hardness, TDS, BOD, COD, SO_4, Ca Mg, Pb, Cd etc. However, appreciable amount of mineral nutrients such as Ca, Fe, Mg, Zn, Cu, and Mn are also present in the textile effluent (Kaushik et al. 2005; Singh et al. 2015) making it a possible source of fertilizer. The fertilization property of textile effluent is well discussed in our earlier published report (Singh and Rathore 2018; Singh et al. 2020). Textile effluent used in the present study was found to be rich in various minerals and trace elements required for the plant physiological activity. Used textile effluent helped to increase the plant growth by providing organic matter and micronutrients in the limited range at lower concentration (Yaseen et al. 2017). However, high COD, total solids, total dissolved solids, soaring alkalinity with higher value of chloride and sulphate can make soil alkaline and reduce availability of micronutrients. Results of the present study indicate that fertilization with textile effluent at low dilution increased the germination property, growth and metabolites of chilli cultivars.

The present experiment showed positive response of textile effluent on germination. Kaushik et al. (2005) and Khan et al. (2011) also observed increase in germination of wheat, pea, lentil and gram with low dilution of textile effluent, and a decrease in seed germination with increase in the concentration of the effluent. Results showed higher seed germination compared to control for all used dilutions of textile effluent (up to 60% dilution) in cultivar GVC-101; however, germination of cultivar GVC-121 was increased up to 40% dilution and was reduced with 60% dilution compare to control. Kaushik et al. (2005) and Khan et al. (2011) suggested that the industrial effluent with high osmotic pressure can cause reduction in germination. However, in case of chilli cultivars, the textile effluent was found to break the dormancy as seen in the present experiment. Hassan et al. (2013) studied the growth of country bean (*Lablab niger* cv. typicus Medikus) seeds and seedlings irrigated with textile effluent. They concluded that the neutralized effluent water does not have a negative impact on the germination percentage, germination energy, relative germination rate and relative effluent injury rate of country bean seeds, or its seedling growth. However, the germination percent varied with cultivars of the same species as seen in this experiment. The maximum promoting effect on germination percent was observed with 10% dilution of textile effluent in both cultivars.

In the present study, germination speed, peak value and germination value also followed the same trend as seed germination. Speed of germination is maximum at 10% dilution and was reduced with increase in textile effluent concentration. The reason for the germination inhibition at higher concentration of textile effluent can be explained by the toxic effect of heavy metals and persistent organic compounds present in the composite textile effluent (Singh and Rathore 2019). The Delay index calculated in the present experiment also showed delayed germination at higher dose of textile effluent, while the Vigor index showed vigorous characteristics of used seed at moderate dose of textile effluent. This result is consistent with Ramana et al. (2002) in vegetable crops (tomato, cucumber, bottle gourd etc.) under distillery effluent.

Plant height and leaf area of chilli cultivars (GVC-101 and GVC-121) under textile effluent fertilization were also increased over control plants. However, fertilization at lower dose textile effluent provided more persistent results as compare to high dose. Similarly to the present study, Panda et al. (2016) also demonstrated that the lower concentrations of industrial effluents promote seed germination, seedling growth and dry matter accumulation of *Oryza* *sativa*. Ajouri et al. (2004) explained that the lower growth in control plants (without treatment) may be due to nutrient deficiency in soil or unavailability to the plants. Deficiency of nutrients in the soil suppress the growth of plants while accumulation of salts such as Cd that could interfere with the uptake of various nutrient elements, decrease root respiration and inhibit root production (Bhuiyan et al. 2016). Fahim et al. (2019) reported improved nutrient content and soil fertility after addition of pulp and paper mill waste which resulted into increased growth and biomass accumulation of *Abelmoschus esculentus* and *Mentha sachalinensis*, Contrary to the results obtained in the present study, Marwari and Khan (2012) reported reduction in root and shoot length by textile effluent.

Deficiency of Zn causes strong chlorosis and decreases leaf production besides the reduction of crop growth. Increase in leaf area can be correlated with increased photosynthetic surface, and thus, higher production (Noulas et al. 2018). Faster development of leaf size and increase in total photosynthetic rate could lead to a general increase of carbon assimilation as evident in the present experiment by increased plant biomass. Similar results were also obtained by Kaushik et al. (2005) on growth and biomass of wheat cultivars irrigated with textile effluent, and by Araújo et al. (2007) on growth and development of soybean and cowpea treated with textile sludge. However, excess heavy metals developed interferences with synthesis of levulinic acid and protochlorophyllide reductase in tetrapyrrole pathway (Wu et al. 2018), resulting into slow rate of photosynthesis (Shah et al. 2017), which further resulted into less biomass accumulation, as also seen with higher dose of textile effluent fertilization in the present experiment.

Chlorophyll estimation is one of the important plant parameters which are used as an index of production capacity of the plant; carotenoids act as an accessory pigment in photosynthesis. Increase in chlorophyll could be due to addition of nutrient by textile effluent fertilization while high concentration of micronutrient showed synergistic effect on either chlorophyll synthesis pathways or on enzymes used for synthesis. Srivastava and Sahai (1988) supported the view that 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 present in the textile effluent. Decreased foliar chlorophyll of plants grown without fertilization in the present study was in accordance with Shah et al. (2017) under nutrient deficiency.

Total carbohydrate and protein content showed similar trend as chlorophyll and increased with age and textile effluent fertilization. Increase in carbohydrate with age may be expected, as starch is converted to carbohydrate as the plants mature (Badoni et al. 2016). Although, suppression of carbohydrate content by high concentration of composite textile effluent can be explained by the presence of high amount of metals and the role of carbohydrate in the enzymatic reactions related to the cycles of carbohydrate catabolism during reactive oxygen species (ROS) generation. Similar to our result, Badoni et al. (2016) also reported an increased accumulation of carbohydrate with increasing concentration of Zn compared to the control in *Jatropha curcas*. Amino acid is the basic precursor for proteins taking part in photosynthesis and photosynthetic pigments, and leaf protein content may positively correlated with biomass and total chlorophyll content of plant (Ayyasamy et al. 2008).

Increase in protein under textile effluent fertilization was seen with lower dose. However, gradual decrease of protein content at higher concentration of textile effluent (above 20% to 60%) suggested the breakdown of protein in amino acid due to stress generated in the presence of the toxic concentration of heavy metals in the textile effluent. However, as explained by Rehman and Bhatti (2009), the enhancement in leaf protein exposed to lower concentration of textile effluent is due to synthesis of stress protein gradually from 30 DAS up to 120 DAS.

Increasing chlorophyll and soluble protein content at lower concentration of effluent application agreed with Gupta and Mittal (2017) and is due to the addition of minerals and nutrients.

Proline is a stress amino acid synthesized according to the defensive capability of plants. The accumulation of proline in plant tissue increases due to stress generated during the growth phases (age of the plant) or under different environmental conditions, i.e., heavy metals, UV light, drought, air pollution (Agrawal et al. 2004; Rathore and Chaudhary 2019; Singh and Rathore 2019; Rathore and Chaudhary 2021). Stress generated from nutrient deficiency in the soil can also cause proline accumulation in the plant (Arias-Baldrich et al. 2015) which can be seen at high concentration of proline in control plants (without textile effluent fertilization). In the present study, proline was least under fertilization with lower dose of textile effluent, suggesting low nutrient stress condition; however, increased proline in T4 might be due to the excess heavy metals and salt added into soil at higher textile effluent application.

5 Conclusions

Textile effluent is rich in nutrients for plants, surplus trace elements and high salt content. Positive response of lower dose (with 20% dilution) of textile effluent fertilization on germination, growth and metabolites of chilli cultivars represented mineral utilization from the effluent. Mineral deficiency or excess mineral developed stress symptoms in plants which can be evident by lower biomass development or higher proline accumulation, as seen in the present study with no fertilization or higher textile effluent fertilization. Lower dose of textile effluent fertilization accumulated least proline and was proved as a most suited condition for growth of the two chilli cultivars. Intra-specific variation among the chilli cultivars GVC-101 and GVC-121 to textile effluent was not evident, although slightly higher efficiency was seen in cultivar GVC-101 compared to cultivar GVC-121 in nutrient utilization from textile effluent. The study concluded that the lower dose of textile effluent can be applied as mineral nutrition supplement for plant growth. However, more field studies are needed to scale up the use of textile effluent, dose appropriation and to asses intra-specific variation.

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Author Contributions RS carried out the experiment, recorded the data, interpreted the result, and wrote the final manuscript. DR designed and supervised the experiment, made suitable changes in the manuscript. Both authors read and approved the final manuscript.

Data Availability Not applicable.

Declarations

Ethics Approval and Consent to Participate Not applicable.

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Affiliations

Ratan Singh[1] · **Dheeraj Rathore**[1]

✉ Dheeraj Rathore
dheeraj.rathore@cug.ac.in

1 School of Environment and Sustainable Development, Central University of Gujarat, Sector-30, Gandhinagar, Gujarat 382030, India