



Improving industrial wastewater irrigation: the effects on Indian baby pumpkin (*Praecitrullus fistulosus*) cultivation

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Abstract

Increasing insufficiency of freshwater resources is driving many countries in arid and semi-arid regions to use low-quality water for agriculture and related activities. This wastewater may have some useful as well adverse effects on various parameters. In this regard, we design an experimental setup to investigate the impact of irrigation with industrial wastewater on the *Praecitrullus fistulosus* (Indian baby pumpkin) plant. For this purpose, *Praecitrullus fistulosus* was cultivated in soil irrigated with different concentrations (*i.e.* 25, 50, 75, 100%) of industrial wastewater, while control contain 0% of wastewater. Before the treatment, physicochemical parameters (pH, TDS, EC, etc.) of industrial wastewater were analysed. It was observed that lower concentration/s of wastewater positively affects various morphological (no. of leaves, fruits etc.) and biochemical (Total protein, Flavonoids, Sugar/s content etc.) attributes in treated plants, whereas elevated concentration may hurt these parameters.

Keywords Improving industrial wastewater · Agriculture activities · *Praecitrullus fistulosus* · Physico- and biochemical parameters

Introduction

In developing countries, effluent water generated by industries is associated with many diseases and shortened life expectancy compared to developed countries (WHO 2002). In Pakistan, only 10% of the wastewater is discharged after the proper treatment, while the rest of the water is discharged into the nearby water bodies without any treatment. On the other hand, increasing insufficiency of freshwater resources is driving to use of low-quality water for agriculture and related activities. In many aspects, this water is not useful for agriculture processes, because it may contain various toxic metals/compounds etc., although it may also have considerable amounts of N, P, K, Ca and other essential nutrients that may serve as a good fertilizer (Riordan 1983; Raman 2002; Saravanamoorthy 2007). Therefore, industrial effluents for irrigation have emerged as an important way of utilizing wastewater. Industrial effluents are considered a low-cost nutrient-rich water source and are used to increase agriculture production (Al-Gheethi 2018). On the other hand, the continued use of industrial wastewater for irrigation causes deterioration of soil as well as has damaging effects on crops and produced quality problems associated with health (Esmailyan 2008; Munir and Ayadi 2004).

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The wastewater may also have heavy metal contamination, and the absorption and accumulation of high concentrations of these heavy metals are considered a hazard to humans and animals feeding on these crops (Sunzid 2020; Adnan 2010). The accumulation of heavy metals in plant tissues might cause a reduction in physiological and biochemical activities, resulting in lower biomass and yield (Scoccianti 2006). Furthermore, these heavy metals enter into the food chain of humans and animals and cause health hazards (Chandran 2012).

Indian baby pumpkin (*Praecitrullus fistulosus*) locally called tinda is a herbaceous plant belonging to the family *Cucurbitaceae* (Tindall 1983; Tyagi 2012). Fruit and seeds of Indian baby pumpkin are used as food, fodder and used for medication for diabetes (Mukesh 2010). Its leaves are also used for maintaining blood pressure (Ciali 2006). The fruit is used as a cooked vegetable. Among the group of medicinal plants, the Indian baby pumpkin is an excellent plant having the configuration of all the essential nutrients best for health (Kirtikar 1998). Indian baby pumpkin is a rich source of carbohydrates, proteins, fibre and fats. It also contains copper, nickel, zinc, lead, cobalt, cadmium, iron, chromium, calcium and sodium contents in trace amounts (Hussain 2010; Holland 1991).

Due to enormous local as well as export market demand, Indian baby pumpkin is one of the highly cultivated vegetables in Pakistan.

Therefore, a study was designed to monitor the effect of industrial wastewater for the cultivation of Indian baby pumpkin (*Praecitrullus fistulosus*) by mixing different concentrations of wastewater with freshwater to find the effects and usefulness of mixed wastewater for agriculture purposes.

Materials and methods

Indian baby pumpkin (*Praecitrullus fistulosus*) seeds were collected from a registered Hyderabad seed corporation company, in Hyderabad Pakistan. The experiments were conducted from March–October at the experimental field of the Institute of Biotechnology and Genetic Engineering (IBGE) in a randomized complete block design (RCBD) manner and experiments were repeated twice. After thirty days of germination, the plants were treated with different concentrations (25, 50, 75 and 100%) of industrial wastewater while the control have 0% of wastewater.

Physicochemical analysis of wastewater

TDS, E.C., salinity and pH were analysed by Orion 5 Star metre, total hardness, chlorides and alkalinity were analysed by titrimetric method, while total phosphate and nitrate

analysed were analysed by Ascorbic acid and Brucine method, respectively (Kalsoom 2015).

Determination of chlorophyll a, b (Chl a, b), and carotenoids

Chlorophyll a, b and carotenoids were estimated by Hartmut and Lichtentaler (1983) method. 50 mg of leaf samples and 5 ml of acetone were taken in test tubes and marked according to the concentration of wastewater and keep this material in the refrigerator for 48 h. The absorption was monitored by Uv–Vis spectrophotometer at wavelength 662, 644 and 470 nm for chlorophyll a, b and carotenoids, respectively.

$$\text{Chlorophyll a (Ca)} = 11.75_{A662} - 2.350_{A644}$$

$$\text{Chlorophyll a (Cb)} = 18.61_{A644} - 3.960_{A662}$$

$$\text{Carotenoids (Cc)} = 1000_{A470} - 2.270 \text{ Ca} - 81.4 \text{ Cb} / 227.$$

Extraction of biochemicals from shoots leaves and fruits

The different parts of the plant (leaves, shoots, and fruits) were completely dried at room temperature. The dried parts were crushed and taken into 70% methanol and centrifuged for 40 min at 6000 rpm. The supernatant was collected and stored at -40°C for further analysis.

Quantification of biomolecules

Total protein from the shoots, leaves and fruit extracts was analysed by Lowry's (1951) method. Total sugar concentration was determined by Montgomery (1961) while reducing sugars were determined by Miller's 1959 procedure. Total phenolic and flavonoid contents were estimated by Yasoubi (2007), and Kim (2003) methods, respectively. Antioxidant activity was quantified by Prieto's (1999) method.

Results and discussion

Physicochemical analysis of industrial wastewater

Before the study of the effects of wastewater on *Praecitrullus fistulosus*, various parameters such as TDS, E.C salinity, pH, total hardness, chlorides and alkalinity were analysed in Table 1. According to the results, the TDS, salinity, total hardness, alkalinity, nitrates and phosphates of wastewater were found 1501, 393 800 and 250 mg/L, respectively, while chlorides 1.2 ppt, and E.C., 2346 ($\mu\text{S}/\text{cm}$) and pH was found 8.40–8.60. The results showed that

Table 1 Physicochemical analysis of industrial wastewater

Parameters	Industrial wastewater
TDS (mg/L)	1501
Salinity (ppt)	1.2
Total hardness mg/L	800
Alkalinity mg/L	250
Nitrates mg/L	2.41
Phosphates mg/L	40.48
Chloride mg/L	393
Conductivity (μ S/cm)	2346
pH	8.40–8.60

Table 2 Metal analysis of industrial wastewater

S.#	Metals	The concentration of metals in industrial wastewater (ppm)
	Iron	5.18
	Zinc	2.68
	Copper	0.25
	Cobalt	0.62
	Nickel	0.21
	Chromium	0.95

this wastewater contained a sufficient amount of nitrates and phosphate which may be useful but the higher values of other parameters like salinity, TDS etc. indicate that the direct use of this water may have some adverse effect on soil and crops.

Metal analysis of industrial wastewater

The concentration of metals in industrial wastewater was determined by atomic absorption spectrophotometer. This water contained various quantities of iron, zinc, copper, cobalt, nickel and chromium Table 2. These metals content may have some beneficial as well as adverse effects; therefore, the concentrated wastewater may not be used directly for agriculture. Especially chromium (VI) species can cause cancer and lung diseases.

Soil analysis

The elemental composition of the soil before and after treatment with wastewater was examined by EDX Figs. 1 and 2. The 100% wastewater has a pronounced effect on oxygen calcium, iron and carbon content, and these contents were found to be reduced while no such prominent effect was observed in other elements Table 3.

SEM analysis of soil

Figure 3 represents SEM images of the soil before and after treatment with wastewater. According to SEM analysis, soil particle size was decreased after treatment of 100% wastewater.

Morphological parameters

In the present study, the Indian baby pumpkin (*Praecitrullus fistulosus*) was treated with different concentrations, i.e. lower 25%, moderate 50% and higher 75 and 100% of industrial wastewater and measured its effect on both morphological

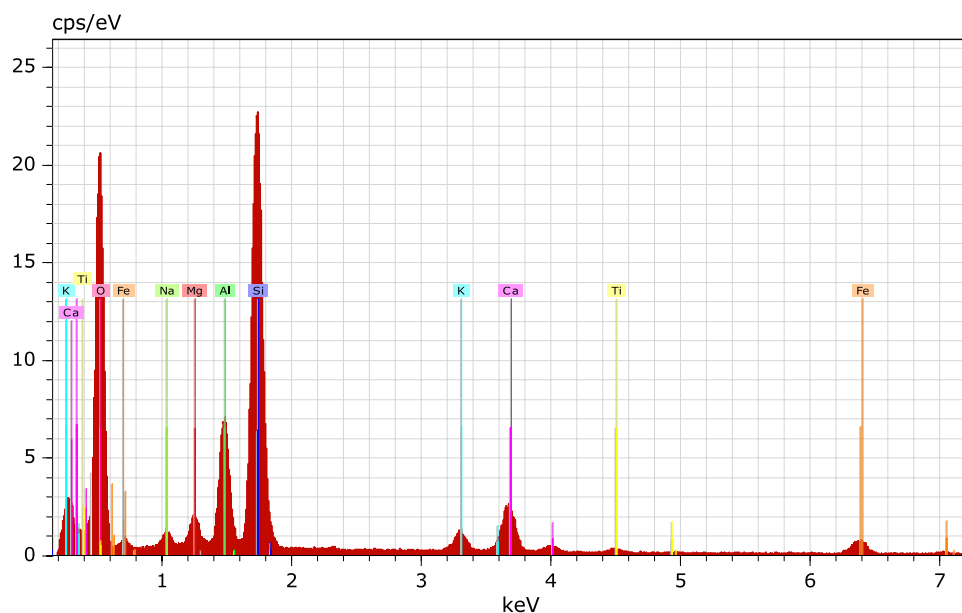
Fig. 1 EDX spectrum of control soil (before treatment of industrial wastewater)

Fig. 2 EDX spectrum of soil treated with 100% industrial wastewater

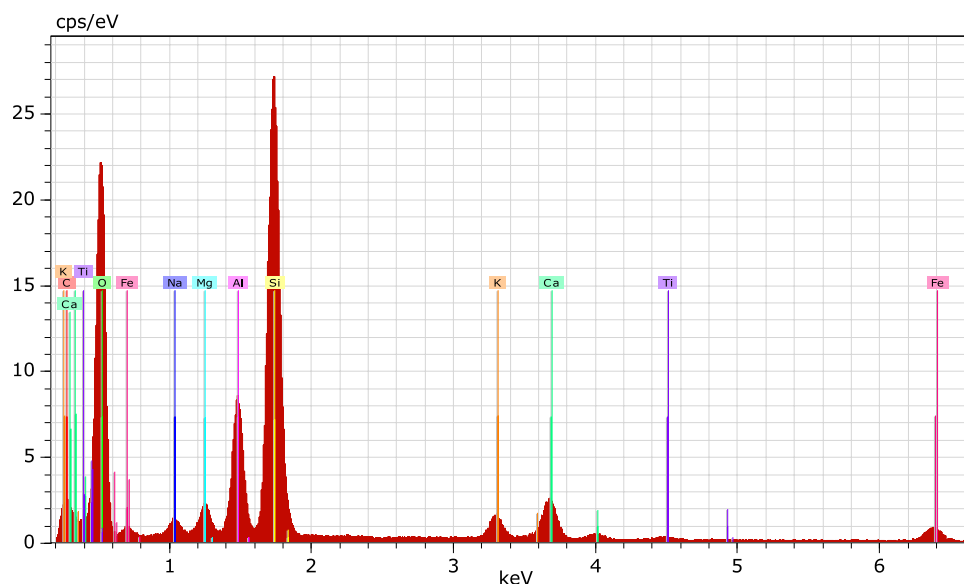


Table 3 EDX analysis of soil before and after treatment

Elemental composition	Soil before treatment (%)	Soil after 100% wastewater treatment (%)
Oxygen	54.35	51.04
Sodium	1.91	1.27
Magnesium	1.64	1.61
Aluminium	6.22	6.73
Silicon	22.58	24.58
Potassium	2.23	2.80
Calcium	6.04	5.04
Titanium	0.75	0.56
Iron	5.02	4.46
Carbon	2.41	1.91

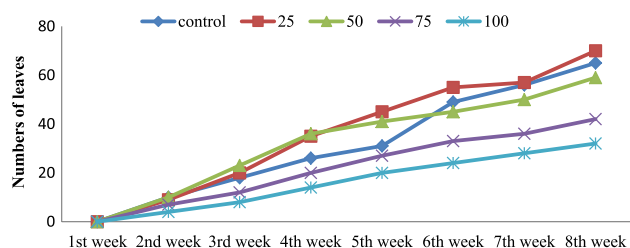


Fig. 4 Effect of industrial wastewater on the number of leaves of Indian baby pumpkin (*Praecitrullus fistulosus*)

and biochemical parameters. These results were compared with the control, containing 0% of wastewater. It was observed that various concentrations have different effects on plant morphology and biochemistry. The morphological parameters such as of the number of leaves and fruits were affected negatively after the treatment with higher concentrations and fewer numbers of leaves and fruits were observed, although lower as well as moderate concentrations give a positive effect on these parameters. It was observed that there was no significant difference between control and treated plants, in terms of the number of leaves and fruits even the plant treated with lower concentration were found to have greater numbers of leaves and fruits than control plants. All these results are summarized in Figs. 4 and 5.

Biochemical parameters

Chlorophyll A, B and total carotenoids

Among different biochemical parameters firstly, chlorophyll a, b and carotenoid contents were analysed and compared with the control. The chlorophyll a, b and carotenoids

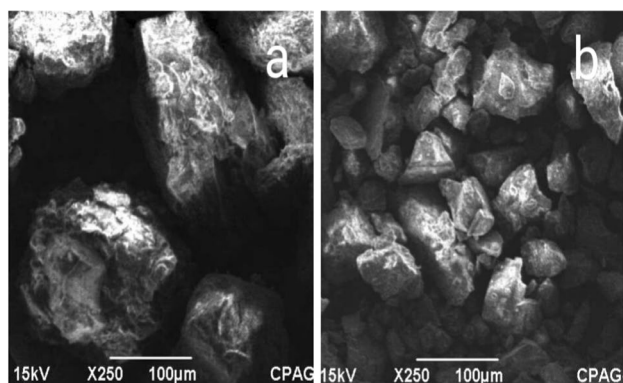


Fig. 3 SEM images of soil **a** before treatment **b** 100% wastewater treated

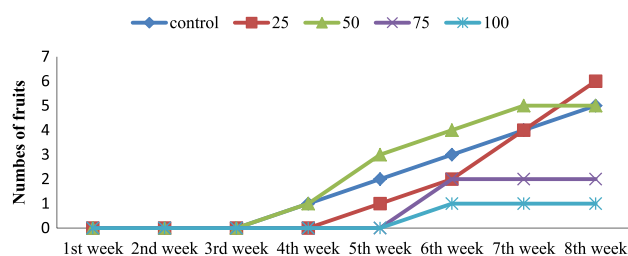


Fig. 5 Effect of industrial wastewater on the development fruits of Indian baby pumpkin (*Praecitrullus fistulosus*)

concentrations were found 14.83, 7.3 and 2.83 mg/g in control plants while 20, 19.89 and 9.18 mg/g for 25%, 19.89, 7.19 and 4.35 mg/g for 50%, 15.28, 5.91 and 3.58 mg/g for 75%, and 7.13, 7.75 and 1.33 mg/g for 100% industrial wastewater-treated plants, respectively Fig. 6. It was observed that the concentration of the above parameter is inversely proportional to the wastewater concentration. The rise in concentration from 25 to 50, 75 and 100% may affect negatively these parameters. Although no such difference was observed in chlorophyll content with increasing concentration from 25 to 50% of wastewater. It showed a more pronounced effect for 75 and 100% of wastewater-treated plants.

Quantification of total protein content

Total protein contents were analysed in shoots, leaves and fruits. The total protein in shoots, leaves and fruits was found 1.17, 2.86 and 3.17 mg/ml, respectively. In comparison with the control, the 25% wastewater-treated plants showed a higher concentration of total protein contents in shoots (1.31 mg/ml), leaves (3.36 mg/ml) and fruits (3.83 mg/ml), while the total protein level declined with increasing the concentration of wastewater. Furthermore, it was also observed that fruits contain higher concentrations of total protein than leaves and shoots. These results are summarized in Fig. 7.

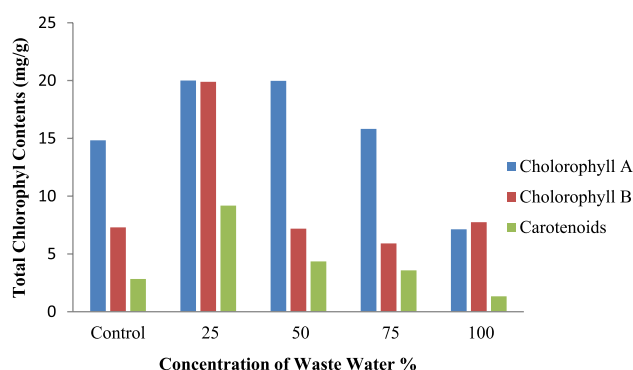


Fig. 6 Effect of industrial wastewater on chlorophyll A, B and total carotenoids of Indian baby pumpkin (*Praecitrullus fistulosus*)

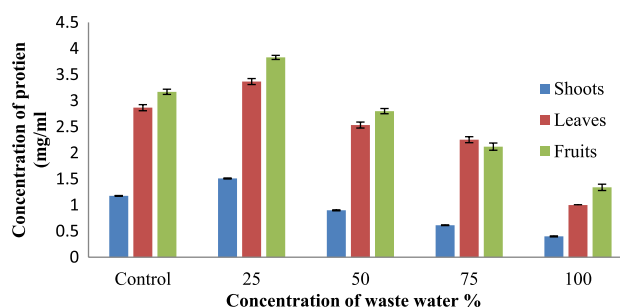


Fig. 7 Effect of industrial wastewater on total protein contents in Indian baby. Pumpkin (*Praecitrullus fistulosus*)

Quantification of total carbohydrates

Total carbohydrate concentration from the shoots, leaves and fruit extracts was determined by Montgomery's (1961) method. The concentration of total carbohydrates in various parts of control plants, i.e. shoots, leaves and fruit extracts, was found to be: (2.21 mg/ml), (2.60 mg/ml) and (2.91 mg/ml), respectively. The elevation was observed in total carbohydrates content in shoots [25% (2.91 mg/ml), 50% (2.52 mg/ml)] even at 75% (2.24 mg/ml)] wastewater-treated plants, although these contents were found to have declined in 100% wastewater-treated plants. The total carbohydrate contents in fruits were found to be increased in 25% of treated plants, whereas these contents were decreased by increasing the concentration of wastewater. In leaves, the suppression of total carbohydrates contents was observed for all concentrations (25, 50, 75, 100%). These results are shown in Fig. 8.

Quantification of reducing sugar

All wastewater-treated plants of Indian baby pumpkin showed low concentrations of reducing sugar in leaves and fruits as compared to control plants' leaves and fruits, in control plant these contents were found in shoots (0.34 mg/ml), leaves (1.46 mg/ml), fruits (1.73 mg/ml), while in 25% treated plants, shoots (1.37 mg/ml), leaves (1.34 mg/ml), fruits (1.53 mg/ml), 50% treated plants, shoots (0.92 mg/ml), leaves (1.23 mg/ml), fruits (1.48 mg/ml), 75% treated plants, shoots (0.86 mg/ml), leaves (0.46 mg/ml), fruits (0.83 mg/ml), and 100% showed lower concentration of reducing sugar than all treated plants, shoots (0.35 mg/ml), leaves (0.45 mg/ml) and fruits (0.35 mg/ml), as shown in Fig. 9.

Total flavonoid contents

The total flavonoid contents in control plants, shoots (0.49 mg/ml), leaves (0.64 mg/ml), fruits (0.77 mg/ml), in 25% treated Indian baby plants, shoots (0.32 mg/ml),

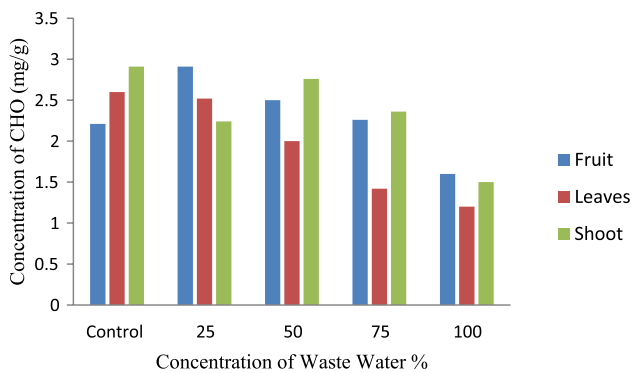


Fig. 8 Effect of industrial wastewater on total carbohydrates in Indian baby pumpkin (*Praecitrullus fistulosus*)

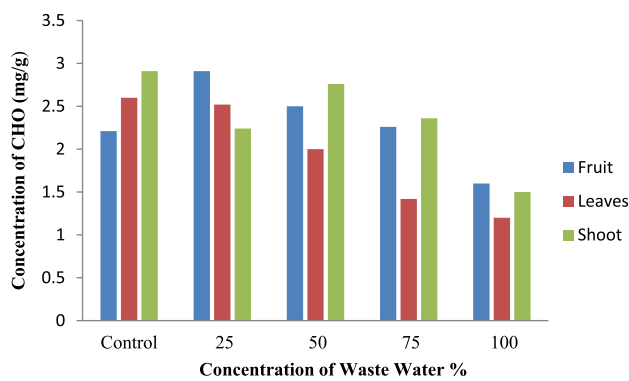


Fig. 9 Effect of industrial wastewater on reducing sugar in Indian baby pumpkin (*Praecitrullus fistulosus*)

leaves (0.64 mg/ml) and fruit (0.77 mg/ml), 50% treated plants, shoots (0.37 mg/ml), leaves (0.37 mg/ml), fruits (0.63 mg/ml), 75% treated plants, shoots (0.19 mg/ml), leaves (0.23 mg/ml), fruits (0.44 mg/ml), and 100% treated plants, shoots (0.21 mg/ml), leaves (0.12 mg/ml) and fruits (0.24 mg/ml) are found in Fig. 10. It was observed that a lower concentration (25%) of wastewater gives a significant effect on flavonoid contents, especially in fruits and leaves although a decline was seen in the case of shoots, while these contents were suppressed in all other cases, i.e. 50, 75 and 100%.

Determination of total phenolics contents

In 25% wastewater-treated plants, the total phenolics were found in shoots (4.54 mg/ml), leaves (7.8 mg/ml) and fruits (8.56 mg/ml) while the control plants contained (4.16 mg/ml), (7.7 mg/ml) (8.36 mg/ml) in shoots, leaves and fruits, respectively. A slight elevation was observed in total phenolic contents in the case of 25% treated plants than control

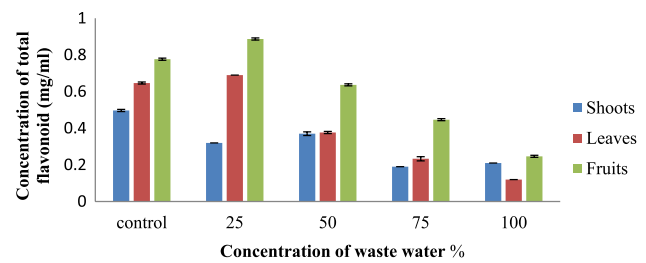


Fig. 10 Effect of industrial wastewater on total flavonoid in Indian baby pumpkin (*Praecitrullus fistulosus*)

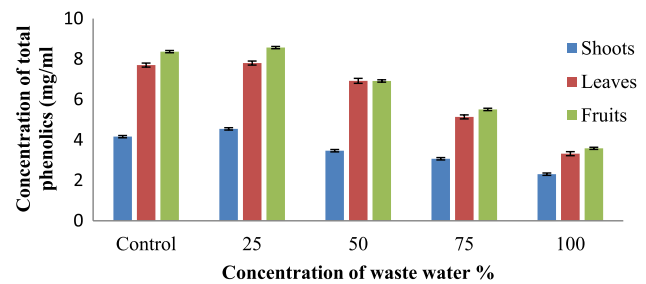


Fig. 11 Effect of industrial wastewater on total phenolics in Indian baby pumpkin (*Praecitrullus fistulosus*)

plants while it was a decline with the rise up concentration of wastewater Fig. 11.

Determination of antioxidant activity

The antioxidant activity of shoots, leaves and fruits was quantified by the Prieto et al. method. Among them, all the parts (shoots, leaves and fruit) showed excellent antioxidant activity, especially fruits obtained by the treatment of 25% wastewater give remarkable antioxidant activity and it was found higher than the control plants. This might be due to high flavonoid and total phenolic contents which are directly related to antioxidant activity. In 50% of wastewater-treated plants, only fruits showed good activity while in the case of shoots and leaves the activity was found to be lower than in control and 25% of wastewater-treated plants. The high concentration of wastewater (75, 100%) gives a negative impact on antioxidant activity on all parts of the plant (shoots, leaves and fruits) Fig. 12.

Conclusion

In conclusion, these experimental results unveiled the progressive effect of irrigation with treated industrial effluent on the growth and biochemical parameters of Indian Baby pumpkins without affecting the quality of various parts of plants. It was observed that the lower or moderate

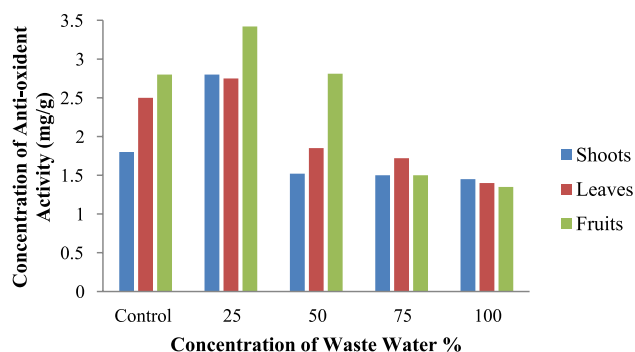


Fig. 12 Effect of industrial wastewater on antioxidant activity in Indian baby. Pumpkin (*Praecitrullus fistulosus*)

concentrations (25 and 50%) of wastewater give a positive response, while higher concentrations (75 and 100%) of wastewater may give a negative effect on the plant growth as well as their biochemical parameters. These effects are directly concerned with the various parameters of wastewater described in the above sections. Therefore, it is of prime importance to emphasize that this statement must be limited to the chemical composition of the treated effluent used and to the Indian Baby pumpkin cultivars tested. Hence, it is recommended that only after proper treatment it can be used for irrigation purposes.

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Declarations

Conflict of Interest There is no conflict of interest.

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