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Effect of Pharmaceutical Effluent on Morphological Parameters and Chlorophyll Content of *Cicer arietinum* and *Vigna radiate*

Bafna Angoorbala and Rathi Iti
Dept. of Biochemistry, Govt. Holkar Science College, Indore, Madhya Pradesh, INDIA

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Abstract

Experiment was conducted to analyse whether pharmaceutical effluent used to irrigate crops is safe or not. Germinated seedlings of Cicer arietinum (black gram) and Vigna radiata (moong) were treated with varying dilutions of untreated pharmaceutical effluents (100 %, 75%, 50%, 25% and 0%) and their effect was calculated on various parameters like viability %, germination%, vigour index, root length, shoot length, dry weight, fresh weight and chlorophyll. From the results of experiment, it was observed that highest reduction in viability % (6%), germination% (13.89%), vigour index (16.01%), root length (16.95%), shoot length (26.50%), fresh weight (22.33%), total chl (36.36%), chl a (28.57%), chl b (37.57%) was obtained at 0% dilution of pharmaceutical effluent while the highest reduction in dry weight (13.11%) was at 75% of pharmaceutical effluent in Cicer arietinum. In case of Vigna radiata the highest reduction in viability % (5%), germination% (2.78%), vigour index (41.74%), root length (38.26%), shoot length (42.06%), fresh weight (22.33%), dry weight (41.17%), chl a (28.57%), chl b (37.67%) was obtained at 0% dilution of pharmaceutical effluent while in case of total chl (20%) maximum reduction was found at 75%.The results of present study showed that pure pharmaceutical effluent is not safe for irrigation but in case of scarcity of water, pharmaceutical effluent could safely be used for irrigation purpose after proper processing and dilution.

Key words: Cicer arietinum, vigna radiata, pharmaceutical effluent, viability %, vigour index.

Introduction

Growth of population, massive urbanization, rapid rate of industrialization and introduction of modern technology in agriculture and animal husbandry lead to water pollution which subsequently results in gradual deterioration of quality of water. The wastes or effluents from urban areas and industries are largely disposed of in rivers, streams and lakes, which are otherwise chief sources of water for agriculture, industries, aquiline life apart from for the domestic purposes[1].

The most potential and hazardous source of water and soil pollution are industrial effluents. These contain heavy metals, poisonous compounds and nutrients, which affect plant and soil in number of ways[2]. Toxic chemicals present in effluents like cyanides, chlorine, hypo chlorites, phenols and heavy metals, caused reduction in cell activities, retardation of growth, various deficiencies and diseases when accumulated in cells of living being[3].

Extensive studies have been done to reflect the effect of industrial effluents on growth and yield parameters of agricultural crops and soil properties. Hence considering all the good and bad effects of industrial effluents on crop plants, the present study was conducted by using effluents obtained from pharmaceutical industry as a source of water to germinate seedlings of two cereal crops i.e. *Cicer arietinum* and *Vigna radiata* and its effect was observed on morphological characters and chlorophyll content.

Material and Methods

The effluent of pharmaceutical industry producing folic acid was used for the present study. The five different dilution of pharmaceutical effluent viz 100%, 75%, 50%, 25% and 0% were used in the study. Healthy seeds of *Cicer arietinum* and *Vigna radiata* were sterilised with 0.1% $HgCl_2$ for 5 min and then were washed thoroughly with distil water. The seeds were then allowed to germinate for 24 hrs. Germinated seeds were then transferred in petriplates lined with Whatmann filter paper no.1.3 ml of respective dilution was added to moisten filter paper in each petriplates and every day at the first day of experiment and then 2 ml of respective dilution was added for consecutive 6 days. Three sets in each dilution were maintained along with the control for comparison. On the seventh day, various growth parameters and chlorophyll content were evaluated as follows:

Germination percentage: The formula given by Rehman *et al.*[4]was used to estimate germination percentage.
Germination % = no. of seeds germinated/ total no. of seeds × 100

Root and shoot length: Length of root and shoot of seedlings were calculated using the standard centimetre scale[3].

Vigour index: The formula suggested by Abdul-Baki and Anderson[5] was used to calculate vigour index.
Vigour index = germination % × (root length +shoot length)*
(* indicate that length of root and shoot in cm).

Fresh and dry weight: Four seeds of each treatment were weighed in order to determine the fresh weight and then dried in oven at 80 ˚C for 24 hrs to obtain dry weight[3]. Fresh weight and dry weight were recorded in gms.

Viability percentage: The method given by Lakon [6] was used to calculate viability percentage. In this method colourless Triphenyl Tetrazolium dye was used which turns red when reduced by respiring embryo i.e. indication of germination.

Chlorophyll estimation: The estimation of chlorophyll content was done according to the method given by Sadasivam and Manickam[7]. Chlorophyll is extracted in 80% acetone and the absorbance at 663nm and 645nm are read in a spectrophotometer. Using the absorption coefficients, the amount of chlorophyll is calculated.

$$\text{Chlorophyll (a) in mg/g tissue} = 12.7(A_{663}) - 2.69(A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll (b) in mg/g tissue} = 22.9(A_{645}) - 4.68(A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll (mg/g tissue)} = 20.2(A_{645}) + 8.02(A_{663}) \times \frac{V}{1000 \times W}$$

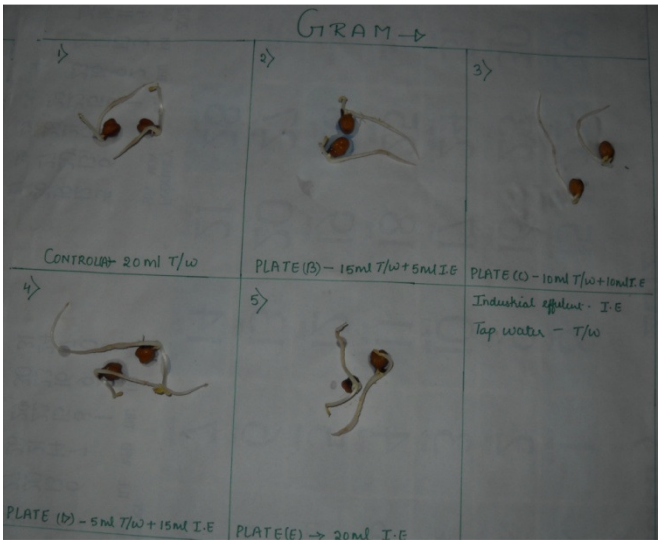

Figure-1
Showing effect of pharmaceutical effluents on root length and shoot length of *Cicer arietinum* seedlings

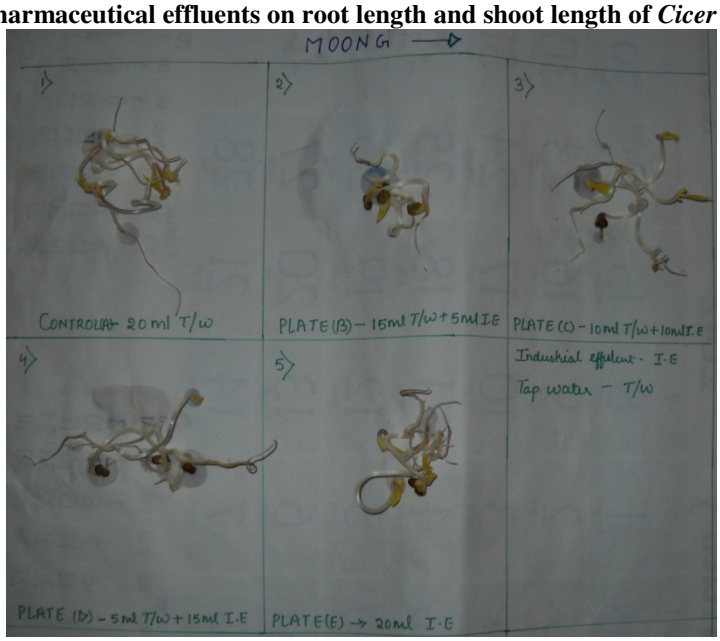

Figure-2
Showing effects of pharmaceutical effluents on root length and shoot length of *Vigna radiata*

Table-1
Showing effect of pharmaceutical effluent on various parameters in *Cicer arietinum*

S.No	Parameters studied	Dilution (%)				
		100%	75%	50%	25%	0%
1	Viability%	100±0	98.66±2.30 ns	95.33±4.16 ns	94.66±5.03 ns	94±5.29 ns
2	Germination%	96±4.00	88±2.00 ns	85.33±9.23 ns	85.33±4.61 ns	82.66±8.32*
3	Vigour Index	887.573±124.12	874.747±65.31 ns	808.693±154.51 ns	807.333±144.46 ns	745.467±45.06 ns
4	Root length(cm)	6.84±1.05	6.30±1.30 ns	5.70±1.31 ns	5.69±0.82 ns	5.68±1.36 ns
5	Shoot length (cm)	3.81±0.74	3.54±0.44 ns	3.50±0.29 ns	3.43±1.32 ns	2.80±0.54 ns
6	Fresh weight (gms)	1.97±0.08	1.75±0.05**	1.61±0.03**	1.54±0.03**	1.53±0.01**
7	Dry weight (gms)	0.61±0.04	0.53±0.01 ns	0.55±0.01 ns	0.56±0.04 ns	0.59±0.02 ns
8	Total chl (mg/g)	0.22 ±0.00	0.20±0.00 ns	0.19±0.01**	0.14±0.01**	0.14±0.01**
9	Chl a (mg/g)	0.07±0.0050	0.06±0.0048 ns	0.05±0.0078 ns	0.05±0.0097 ns	0.05±0.0048 ns
10	Chl b (mg/g)	0.1485±0.0036	0.1356±0.0061 ns	0.1343±0.0099**	0.0930±0.0097**	0.0927±0.0144**

*= Values are Significant (p<0.05),**= Values are very significant (p <0.01) and ns= not significant (p>0.05).

Table-2
Showing effect of pharmaceutical effluent on various parameters in *Vigna radiata*

S.No	Parameters studied	Dilution (%)				
		100%	75%	50%	25%	0%
1	Viability%	100±0	96.66±5.77 ns	96.66±5.77 ns	96.66±5.77 ns	95±5.00 ns
2	Germination%	96±4	94.66±6.11 ns	94.66±4.61 ns	94.66±6.11 ns	93.33±2.30 ns
3	Vigour index	905.62±86.58	813.37±3.17 ns	629.06±147.34**	629.06±123.41**	527.58±128.84**
4	Root length(cm)	3.58 ±0.18	3.40±0.14 ns	2.99±0.52**	2.83±0.54**	2.21±0.67**
5	Shoot length (cm)	5.83±0.39	5.17±0.35 ns	3.86±0..88**	3.67±0.92**	3.38±0..49**
6	Fresh weight (gms)	1.9±0.17	1.89±0.18 ns	1.86±0.40 ns	1.76±0.15 ns	1.60±0.12 ns
7	Dry weight (gms)	0.10±0.02	0.14±0.01 ns	0.15±0.04 ns	0.16±0.04 ns	0.17±0.01 ns
8	Total chl (mg/g)	0.10±0.01	0.08±0.00 ns	0.09±0.00 ns	0.09±0.00 ns	0.09±0.00 ns
9	Chl a (mg/g)	0.07±0.00	0.06±0.01*	0.06±0.01*	0.06±0.01*	0.05±0.00 *
10	Chl b (mg/g)	0.0361±0.1806	0.0307±0.0016 ns	0.0294±0.0030 ns	0.0257±0.0037 ns	0.0225±0.0033 ns

*= Values are Significant (p<0.05),**= Values are very significant (p <0.01) and ns= not significant (p>0.05).

Results and Discussion

Table -1 is showing effect of different dilution% of pharmaceutical effluent on viability %, germination %, vigour index, root length, shoot length, fresh weight, dry weight, total chlorophyll, chlorophyll a and chlorophyll b in *Cicer arietinum*.

Viability % in untreated seedlings of *Cicer arietinum* was 100±00%while the viability % of seedlings treated with 75%, 50%, 25% and 0 % dilution were 98.66±2.30, 95.33±4.16, 94.66±5.34 and 94±5.29 respectively. The maximum reduction was (6%) found at 0% dilution.

Germination % in untreated seedlings of *Cicer arietinum* was 96%.while the germination % of seedlings treated with 75%, 50%, 25% and 0 % dilution were 88±2, 85.33±9.23, 85.33±4.61 and 82±8.32 respectively. The maximum reduction was (13.89%) found at 0% dilution.

Vigour index in untreated seedlings of *Cicer arietinum* was 887.573while the vigour index of seedlings treated with 75%,

50%, 25% and 0 % dilution were 874.747±65.31, 808.693 ± 154.51, 808.333 ± 144.46 and 745.467 ± 45.06 respectively. The maximum reduction was (16.01%) found at 0% dilution.

Root length in untreated seedlings of *Cicer arietinum* was 6.84±1.05 while the root length of seedlings treated with 75%, 50%, 25% and 0 % dilution were 6.30±1.30, 5.70±1.31, 5.69.±0.82 and 5.68±1.36 respectively. The maximum reduction was (16.95%) found at 0% dilution (figure 1).

Shoot length in untreated seedlings of *Cicer arietinum* was 3.81±0.74 while the shoot length of seedlings treated with 75%, 50%, 25% and 0 % dilution were 3.54±0.44, 3.50±0.29, 3.43.±1.32 and 2.80±0.54 respectively. The maximum reduction was (26.50%) found at 0% dilution (figure 1).

Fresh weight in untreated seedlings of *Cicer arietinum* was 1.97±0.08 while the fresh weight of seedlings treated with 75%, 50%, 25% and 0 % dilution were 1.75±0.05, 1.61±0.03, 1.54.±0.03 and 1.53±0.01respectively. The maximum reduction was (22.82%) found at 0% dilution.

Dry weight in untreated seedlings of *Cicer arietinum* was 0.61±0.04.while the dry weight of seedlings treated with 75%, 50%, 25 5and 0 % dilution were 0.53±0.01, 0.55±0.01, 0.56.±0.04 and 0.59±0.02 respectively. The maximum reduction was (13.11%) found at 75% dilution.

Total chlorophyll content in untreated seedlings of *Cicer arietinum* was .22±.00 while the total chlorophyll content of seedlings treated with 75%, 50%, 25% and 0 % dilution were .20±.00, .19±.01, .14.±.01 and .14±.01 respectively. The maximum decrease were (37.57%) found at 25% and 0% dilution.

Chlorophyll a content in untreated seedlings of *Cicer arietinum* was .07±.0050 while the chlorophyll a content of seedlings treated with 75%, 50%, 25% and 0 % dilution were .06±.0048, .05±.0078, .05.±.0097 and .05±.0048 respectively. The maximum decrease were (37.57%) found at 50%, 25% and 0% dilution.

Chlorophyll b content in untreated seedlings of *Cicer arietinum* was .1485±.0036 while the chlorophyll b content of seedlings treated with 75%, 50%, 25% and 0 % dilution were .1356±.0061, .1343±.0099, .0930.±.0097 and .0927±.0144 respectively. The maximum decrease was (37.57%) 0% dilution.

Table-2 is showing effect of different dilution% of pharmaceutical effluent on viability %, germination %, vigour index, root length, shoot length, fresh weight, dry weight, total chlorophyll, chlorophyll a and chlorophyll b in *Vigna radiata*.

Viability % in untreated seedlings of *Vigna radiata* was 100±00% while the viability % of seedlings treated with 75%, 50%, 25% and 0 % dilution were 96.66±5.77, 96.66±5.77, 96.66±5.77 and 95±5.00 respectively. The maximum reduction was (5%) found at 0%.

Germination% in untreated seedlings of *Vigna radiata* was 96±4.while the germination % of seedlings treated with 75%, 50%, 25% and 0 % dilution were 94.66±6.11%, 94.66±6.11%, 94.66±6.11% and 93±2.30% respectively. The maximum reduction was (2.78%) found at 0% dilution.

Vigour index in untreated seedlings of *Vigna radiata* was 905.62±86.58while the vigour index of seedlings treated with 75%, 50%, 25% and 0 % dilution were 813.37±3.17, 629.06±147.34, 629.06±6.123.41 and 527.58±128.84 respectively. The maximum reduction was (41.74%) found at 0% dilution.

Root length in untreated seedlings of *Vigna radiata* was 3.58±0.18 while the root length of seedlings treated with 75%, 50%, 25% and 0 % dilution were 3.40±0.14, 2.99±0.52, 2.83.±0.54 and 2.21±0.67 respectively. The maximum reduction was (38.26%) found at 0% dilution (figure 2).

Shoot length in untreated seedlings of *Vigna radiata* was 5.83±0.39 while the shoot length of seedlings treated with 75%, 50%, 25% and 0 % dilution were 5.17±0.35, 3.86±0.88, 3.67±.92 and 3.38±0.49 respectively. The maximum reduction was (42.02%) found at 0% dilution (figure 2).

Fresh weight in untreated seedlings of *Vigna radiata* was 1.97±0.17while the fresh weight of seedlings treated with 75%, 50%, 25% and 0 % dilution were 1.89±0.18, 1.86±0.40, 1.76±0.15 and 1.60±0.12 respectively. The maximum reduction was (15.78%) found at 0% dilution.

Dry weight in untreated seedlings of *Vigna radiata* was 0.10±0.02while the dry weight of seedlings treated with 75%, 50, 25% and 0 % dilution were 0.14±0.01, 0.15±0.04, 0.16±0.04 and 0.17±0.01 respectively. The maximum increase was (41.17%) found at 0% dilution.

Total chlorophyll content in untreated seedlings of *Vigna radiata* was .01±.01 while the total chlorophyll content of seedlings treated with 75%, 50%, 25% and 0 % dilution were .08±.00, .09±.00, .09.±.00 and .09±.00 respectively. The maximum decrease was (20%) found at 75% dilution.

Chlorophyll a content in untreated seedlings of *Vigna radiata* was .07±.00 while the chlorophyll a content of seedlings treated with 75%, 50%, 25% and 0 % dilution were .06±.01, .06±.01, .06.±.01 and .05±.00 respectively. The maximum decrease was (28.57%) found at 0% dilution.

Chlorophyll b content in untreated seedlings of *Vigna radiata* was .0361±.1806 while the chlorophyll b content of seedlings treated with 75%, 50%, 25% and 0 % dilution were .0307±.0016, .0294±.0030, .0257.±.0037 and .0225±.0033

respectively. The maximum decrease was (37.67%) found at 0% dilution.

Direct discharge of effluents changes the physico chemical and biological characteristics of soils and was responsible for the reduction in the rate of germination of seeds but studies have proved that properly diluted effluents can be used for irrigation[8]. Favourable effects of diluted effluents on seedling growth have been investigated and well documented[9,10].

Free radicals and toxic reactive oxygen species are generated when heavy metals induce oxidative stress. These species cause lipid peroxidation, membrane damage and inactivation of enzymes when they react with lipids, proteins, pigments and nucleic acids and in turn affecting the cell viability. The dangerous effects of oxidative stress may be alleviated by the enzymatic and non enzymatic antioxidant machinery of the plant[11, 12]. Pharmaceutical effluent showed negative impact on viability % of both *Cicer arietinum* and *Vigna radiata* i.e. the viability decreased with decreased in dilution %. Maximum viability was seen at 100% dilution in both *Cicer arietinum* and *Vigna radiata*. In contrary study done *on Vigna unguiculata* showed high viability (71.71%) using effluent from paint, battery and textile[13].

In present study the Germination % in *Cicer arietinum* and *Vigna radiata* decreased with decreased in dilution %. The result was in accordance with the work of some researchers which showed in their experiment that there was 100% germination in wheat in control after 48 hours[14].

The vigour index was significantly decreased in *Vigna radiata* while in *Cicer arietinum* the decreased was not significant with decrease in dilution %. In contradiction to the results of present study, some studies found increased germination and vigour index of rice, maize, black gram, green gram and soybean crops with 25, 50 and 75% pulp and paper mill effluent concentration as compare to the normal water[15].

Fresh weight, dry weight and root and shoot length of seeds are used as indices of growth performance. In present study, *Cicer arietinum* and *Vigna radiata* showed differences in their responses to various dilution % in both root length and shoot length but the decreased was significant in *Vigna radiata* compared to *Cicer arietinum*. The result of present study was supported by some workers who showed that lengths of root and shoot decrease as concentration of effluent increases[14]. Results of some scientists showed similarity with the results of present study and suggested that plumule length is decreasing in higher concentration of polluted water[16].

In present study the fresh weight of both *Cicer arietinum* and *Vigna radiata* was decreased with decreased in dilution %. The reduction was significant in *Cicer arietinum* as compared to *Vigna radiata*. The result was supported by work done by researchers which showed that weights decreases as the concentration of effluent increases[14]. Effluent from a textile industry did not show any inhibitory effect on bio mass of three cultivators of wheat at low concentration (6.25%)[17].

In present study the dry weight of *Cicer arietinum* was decreased while the dry weight of *Vigna radiata* was increased. Some scientist found highest dry weight in control than the effluent treated plants in all the varieties of ground nut[18]. Application of polluted water decreased dry weight[19].

The study result indicates that the content of total chlorophyll, chlorophyll a and chlorophyll b was decreased in both types of seed but the reduction was significant in *Cicer aritenum* as compared to *Vigna radiata*. The result of a study done on cowpea coincides with the result of present study which revealed that the contents of the chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid were found to be decreased at all the treatments over control[20].

Conclusion

It was concluded from the present study that the morphological characters like viability %, germination %, vigour index, root length, shoot length and fresh weight did not show significant effect at any dilution except dry weight which was significantly reduced in *Cicer arietinum*. The biochemical parameters like total chl, chl a and chl b was not significantly affected at 75% dilution while significantly reduced at 50%, 25% and0% in *Cicer arietinum*. In *Vigna radiata* the morphological characters like viability %, germination%, fresh weight, and dry weight was not significantly affected while vigour index, root length and shoot length was not affected significantly at 75% while significantly affected at 50%,25% and 0% .The biochemical parameters like total chl and chl b was not affected significantly while chl a was significantly affected, so it was suggested that undiluted pharmaceutical effluents should be properly processed and diluted before being used for irrigation purpose.

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