Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env.Pharmacol. Life Sci., Vol9[4] March 2020 : 117-123

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Assessment of the Efficiency of *Ceratophyllum demersum* in Waste Water Treatment

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ABSTRACT

Ceratophyllum demersum, commonly known as Coontail or Hornwort is a submerged, free floating aquatic plant. The aquatic plant Ceratophyllum was grown for phytoremediation treatment in range 0 (control), 20, 40, 60, 80 and 100% waste water. Fresh matter yield and dry matter yield of 15, 30 and 60 days growth of Ceratophyllum showed maximum value at 40% level of waste water. Chlorophyll content of 15 days growth of Ceratophyllum was found maximum at 40% level while 20% level of waste water showed maximum chlorophyll content of 30 and 60 days growth of Ceratophyllum plant. Maximum ascorbic acid content and catalase activity was recorded at 60% level of waste water in 15 days, 40% level of waste water in 30 days and 20% level of waste water in 60 days growth of Ceratophyllum plant. Overall, it is concluded that waste water can be used for irrigation of crop plants after phytoremediation treatment with Ceratophyllum demersum aquatic plant.

Keywords: *Ceratophyllum demersum, Waste Water, Phytoremediation, Aquatic Plant*

Received 27.12.2019 Revised 11.02.2020 Accepted 11.03.2020

INTRODUCTION

Water quality assessment in an important activity in agricultural water management. Water irrigation of insufficient quality could retard plant growth and could contaminate soil making it less suitable for agriculture. Phytoremediation is mainly applied to lightly contaminated soils and waters where the material to be treated is low or medium in depth and the area to be treated is large [1]. For both planting and harvesting, phytoremediation makes agronomic techniques economical and applicable [2].

Ceratophyllum demersum, commonly known as hornwort, rigid hornwort, [3] coontail, or coon's tail, [4] is a species of *Ceratophyllum*. It is a submerged, free-floating aquatic plant, with a cosmopolitan distribution, native to all continents except Antarctica. It is a harmful weed in New Zealand [4].

Ceratophyllum demersum, an aquatic plant, has stems that reach lengths of 1–3 m (3–10 ft), with numerous side shoots that make one specimen appear as a large, bushy mass. The leaves are composed of six to twelve whorls, each leaf 8–40 mm long, flat, or forked into two to eight thread-like segments tipped with spiny teeth; they are rigid and brittle. It is monoecious, produced on the same plant with separate male and female flowers. The flowers are tiny, 2 mm long, with 8 or more greenish-brown petals; they are formed in the axils of the vine. The fruit is a 4–5 mm long small seed, typically with three spines, two basal spines and one apical spine, 1–12 mm long [5-10].It can form turions buds that sink to the bottom of the water that stay there during the winter and form new plants in spring.

Several reports indicate that *C. demersum* may exert allelopathic effects on its environment. Most workers have studied the effects of plant extracts and often have not considered whether these allelopathic substances actually leave the live plants and exert their effects in situ.

MATERIAL AND METHODS

Waste water samples were collected from waste water channel of Ganga Barrage, Kanpur in wide large plastic containers and bottle cork immediately. All the samples were brought to settle in an open plastic tubes for one week to allow micro organism to break down solid organic waste and then added to ice cube then transferred to the lab where it was placed in the refrigerator.

COLLECTION OF PLANT

For culture of aquatic plants *Ceratophyllum* and water sample were collected from the pond near Maswanpur, Kalyanpur, Kanpur. After collecting plants in plastic bags with some amount of water from the same site were transported to the laboratory where it were washed with fresh tap water and then finally with distilled water to remove any soil or sediments particles attached to plant surface. The wastewater collected then filtered through multifolded cloths and brought the laboratory and stored in a refrigerator till before treatment analysis was completed. Sample for estimation of dissolved oxygen was collected in 250 ml bottle and fixed immediately of each treatment were analyzed. For the proposed study the test plants *Ceratophyllum* were collected from nearby area of Manawati marg. For culture of *Ceratophyllum demersum* aquatic plant 6" x 9" enamal disc were used. The concentrations of waste water were 0, 20, 40, 60, 80 and 100%. For the control (0%) pond water was used. For study, fresh matter yield, dry matter yield, chlorophyll and ascorbic acid content and catalase activity are recorded at 15, 30 and 60 days growth of *Ceratophyllum demersum*.

Entire data have been statistically analyzed and tested for significance at 5% and 1% probability levels subjected to the analysis of variance according to Steel and Torrie [11]. Fresh matter yield was determined by weight first washing with running tap water, rinsing with distilled water and absorbing surface with clean white blotting paper. Dry matter yield was determined by drying and finely chopped and mixed plants samples in a forced draught oven at 65^{o}C for 24 hours to constant weight. The samples were taken out from the oven and placed in a desiccators, cooled for about an hours and weighed for the determination of yield. For dry matter yield fresh matter kept for drying was thoroughly cleaned against any surface contamination by first washing with running tap water, rinsing with distilled water and absorbing surface water with clean white blotting sheets. Chlorophyll was determined by the method of Petering et al [12] and the chlorophyll content was measured by estimating the absorption of the acetone extract in. Elico-CL- 20A-Photo-electric-calorimeter used red filter and referring the reading to the standard calibration curve prepared by the method of Comer and Zscheile [13]. Ascorbic acid content was estimated titrimetrically by the method of Harris and Roy [14] and Catalase was assayed by the permanganate titration method of the Euler and Josephson [15].

RESULTS

FRESH MATTER YIELD

Upto 40 % increase in waste water level, increase in fresh matter yield of 15, 30 and 60 days growth of *Ceratophyllum demersum* plant was observed. Further increase in waste water level showed decrease in fresh matter yield of 15, 30 and 60 days growth of *Ceratophyllum demersum* plant. As compared to control 20%, 40%, 60% and 80% waste water level showed highly significant (P=0.01) increase in fresh matter yield of 15, 30 and 60 days growth of *C.demersum* plant. 100% wastewater in 15, 30 and 60 days of *Ceratophyllum demersum* plant showed toxic effect for fresh matter yield as compared to control. Increase in fresh matter yield 20% waste water level over control and 40% over 20% waste water level showed highly significant (P=0.01) increase in fresh matter yield of 15, 30 and 60 days growth of *Ceratophyllum demersum* plant. A highly significant (P=0.01) decrease in fresh matter yield of 15, 30 and 60 days growth of *Ceratophyllum demersum* plant was observed at 60% over 40%, 80% over 60% and 100% over 80% waste water level. Maximum fresh matter yield of 15, 30 and 60 days growth of *Ceratophyllum demersum*was observed at 40% level of wastewater.

DRY MATTER YIELD

Increase in dry matter yield of 15, 30 and 60 days growth of *Ceratophyllum demersum* plant was observed with the increase in waste water level upto 40 %. Further increase in waste water level decrease in dry matter yield of 15, 30 and 60 days of *Ceratophyllum demersum* plant was observed. As compare to control, increase in dry matter yield was found to be highly significant (P=0.01) at 20%, 40%, 60% and 80% waste water level in 15, 30 and 60 days growth of *Ceratophyllum demersum* plants. 100% waste water level in 15, 30 and 60 days growth of *Ceratophyllum demersum* showed toxic effect for dry matter yield. Increase in dry matter yield of *Ceratophyllum demersum* plant at 15, 30 and 60 days growth was found to be highly significant (P=0.01) at 40% over 20% and 20% waste water level over control. Decrease in dry matter yield of *Ceratophyllum demersum* plant at 15, 30 and 60 days growth was found to be highly significant (P=0.01) at 60% over 40%, 80% over 60% and 100% over 80% waste water level. 40% wastewater level showed maximum dry matter yield of 15, 30 and 60 days growth of *Ceratophyllum demersum*.

CHLOROPHYLL CONTENT

With the increase in waste water level up to 40 % in 15 days growth and up to 20 % level in 30 and 60 days growth of chlorophyll content of *Ceratophyllum demersum* plant. Beyond this respective level further increase in waste water decrease the chlorophyll content of *Ceratophyllum demersum*. As compared to control 20% waste water in 15, 30 and 60 days growth, 40% and 60% waste water in 15 days growth of *Ceratophyllum demersum*showed highly significant (P=0.01) increase, whereas 40% waste water in 30 days growth showed significant (P=0.05) increase in chlorophyll content of *Ceratophyllum demersum* plant. 80% and 100% waste water in 15, 30 and 60 days growth, 60% waste water in 30 and 60 days growth, and 40% waste water in 60 days growth was found to be toxic for chlorophyll content of *Ceratophyllum demersum* plant over control. Increase in chlorophyll content of *Ceratophyllum demersum* plant was found to be highly significant (P=0.01) at 20% over control in 15, 30 and 60 days growth, and at 40 over 20% waste water level in 15 days growth of *Ceratophyllum demersum* plant. Decrease in chlorophyll content of *Ceratophyllum demersum* plant was highly significant (P=0.01) 60% over 40%, 80% over 60% and 100% over 80% waste water in 15, 30 and 60 days growth, and 40% over 20% waste water in 30 and 60 days growth of *Ceratophyllum demersum* plant. At 40% maximum chlorophyll content of 15 days growth and at 20% level of 30 and 60 days growth was observed.

ASCORBIC ACID

Ascorbic acid of *Ceratophyllum demersum* increased with the increase in waste water level upto 60% at 15 days growth, upto 40% at 30 days growth and upto 20% level at 60 days growth. Beyond these respective levels further increase in waste water level decreased the ascorbic acid content of *Ceratophyllum demersum*. As compared to control increase in ascorbic acid content of *Ceratophyllum demersum* plant was found to be highly significant (P=0.01) at all the level tested in 15 days growth, upto 80% waste water in 30 days growth and upto 40% waste water in 60 days growth of *Ceratophyllum demersum* plant. 100% waste water showed equal value for ascorbic acid content in 30 days growth with control. However, 60% over control showed only significant (P=0.05) decrease in ascorbic acid content in 60 days of plant growth. 80% and 100% waste water showed toxic effect for ascorbic acid content of 60 days growth of *Ceratophyllum demersum* plant over control.

Increase in ascorbic acid content of *Ceratophyllum demersum* plant was found to be highly significant (P=0.0.1) at 20% over control in 15, 30 and 60 days growth, 40% over 20% in 15 and 30 days growth and 60 over 40% waste water in 15 days growth of *Ceratophyllum demersum* plant. 100% over 80% waste water in 15 and 30 days, 60% over 40 % in 30 and 60 days, 40% over 20 % in 60 days, 80% over 60% in 15 and 60 days growth of *Ceratophyllum demersum* (L.) showed highly significant (P=0.01) decrease in ascorbic acid content. Significant (P=0.05) decrease showed at 100% over 80% in 60 days growth of *Ceratophyllum demersum* (L.). Maximum ascorbic acid content of 15 days growth at 60%, 30 days growth at 40% and 60 days growth of *Ceratophyllum demersum* (L.) at 20% level was observed.

CATALASE ACTIVITY

With the increase in waste water level up to 60% at 15 days growth, up to 40% level at 30 days growth and 20% level at 60 days growth of catalase activity of Ceratophyllum demersum (L.) increased. Further increase in waste water level decrease the catalase activity of Ceratophyllum demersum (L.) was observed.As compared to control, increase in catalase activity of Ceratophyllum demersum (L.) was found to be highly significant (P=0.01) at 40%, 60% and 80% waste water in 15 days. 40% waste water in 30 days, and 20% waste water in 60 days growth showed significant (P=0.05) increase. 20% waste water in 15 days, 20%, 60% and 80% waste water in 30 days and 40% waste water in 60 days growth showed insignificant increase while 80% and 100% waste water for 60 days, 100%% wastewater in 30 days growth of *Ceratophyllum demersum* (L.) showed insignificant decrease.

Increase in catalase activity of *Ceratophyllum demersum* (L.) was found to be significant (P=0.05) at 20% waste water over control in 60 days. 20% over control in 15 days, 40% over 20 % in 15 and 30 days, 20% over control in 30 days, 60% over 40% showed insignificant increase in 15 days. Decrease in catalase activity of *Ceratophyllum demersum* (L.) was found in insignificant at 40% over 20 %, 60% over 40%, 80% over 60 % and 100% over 80% in 60 days growth, 80% over 60% waste water in 15 days, 60% over 40% and 100% over 80% waste water in 30 days. Highly significant (P=0.01) decrease at 100% over 80% waste water in 15 days. However, value of 80% and 60% were found equal in 30 days growth.Maximum catalase activity of 15 days growth at 60% level, of 30 days growth at 40% level and of 60 days growth of *Ceratophyllum demersum* (L.) at 20% level was observed.

Table 1: Effect of waste water on Fresh matter yield, Dry matter yield, Chlorophyll content, Ascorbic acid content and Catalase activity of *Ceratophyllum demersum*

Days	Percent Waste Water						LSD	
	C	20	40	60	80	100	P = 0.05	P =0.01
Fresh matter Yield / Treatment (gm)								
15	6.35	10.43	12.20	11.55	9.38	5.80	0.04	0.06
30	7.57	22.30	26.45	24.72	20.44	7.25	0.05	0.07
60	8.96	40.16	43.77	41.80	31.95	8.68	0.04	0.06
Dry matter yield / Treatment (gm)								
15	0.725	1.190	1.394	1.318	1.068	0.662	0.005	0.006
30	0.863	2.545	3.016	2.819	2.334	0.829	0.004	0.006
60	1.021	4.528	4.993	4.770	3.645	0.995	0.004	0.005
Chlorophyll content /g FM (mg)								
15	0.727	0.813	0.870	0.803	0.670	0.583	0.022	0.031
30	0.760	0.870	0.780	0.670	0.533	0.490	0.018	0.026
60	0.787	0.967	0.653	0.520	0.483	0.430	0.018	0.026
Ascorbic acid / g FM (mg)								
15	0.020	0.023	0.027	0.029	0.026	0.022	0.001	0.002
30	0.021	0.024	0.028	0.025	0.024	0.021	0.001	0.002
60	0.023	0.028	0.026	0.022	0.020	0.019	0.001	0.002
Unit Catalase/g FM								
15	1.10	1.30	1.50	1.70	1.60	1.30	0.21	0.29
30	1.30	1.40	1.60	1.40	1.40	1.20	0.23	0.32
60	1.30	1.70	1.40	1.30	1.20	1.10	0.33	0.46

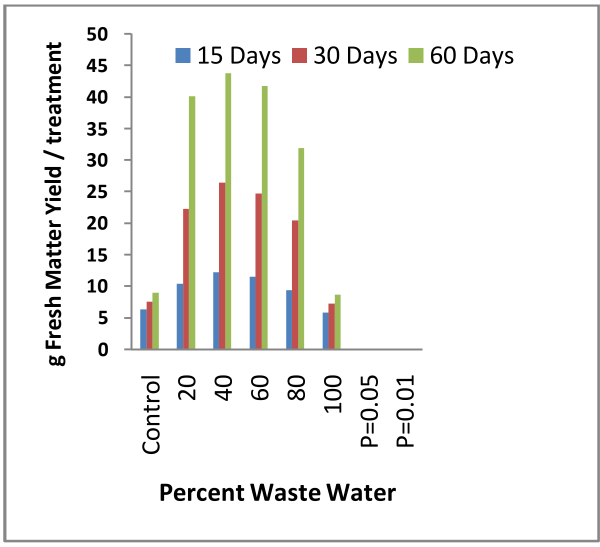


Fig 1: Effect of different conc. of waste water on Fresh Matter Yield

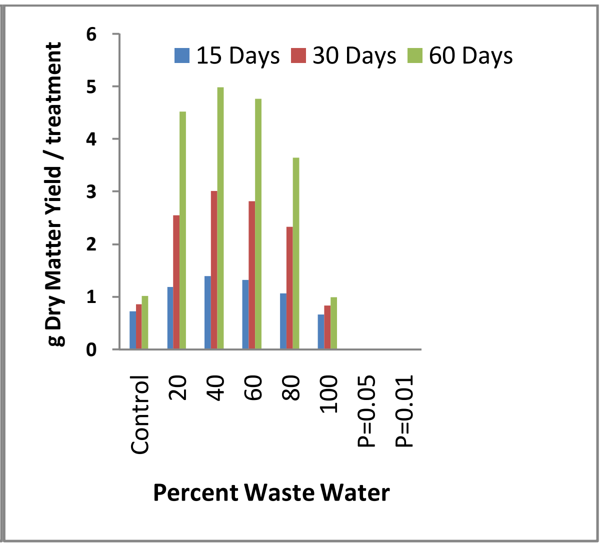
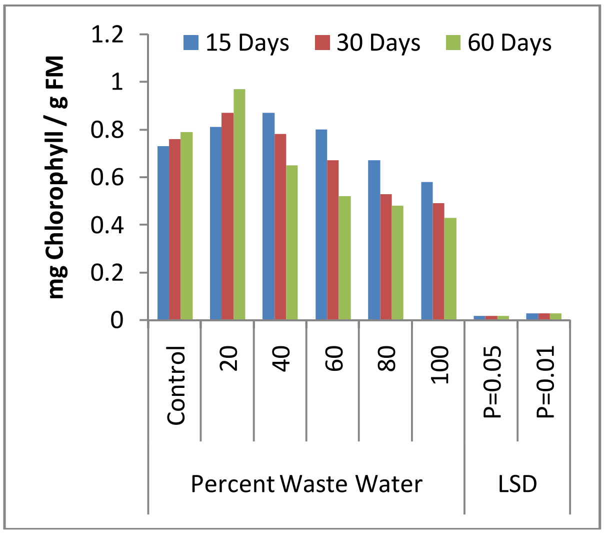


Fig 2: Effect of different conc. of waste water on Dry Matter Yield



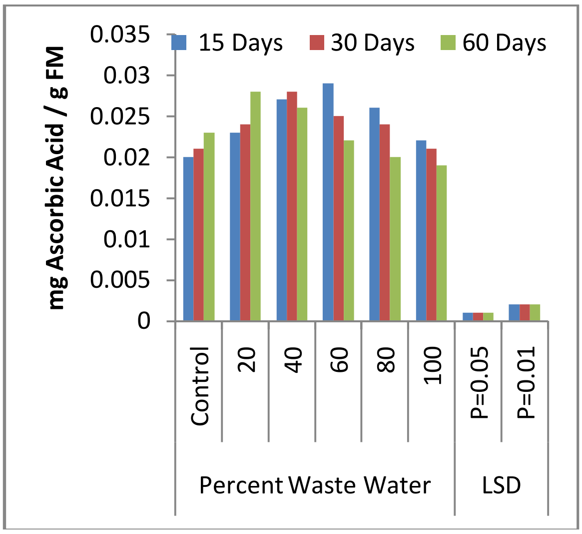


Fig 3: Effect of different conc. of waste water on Chlorophyll Content

Fig 4: Effect of different conc. of waste water on Ascorbic Acid

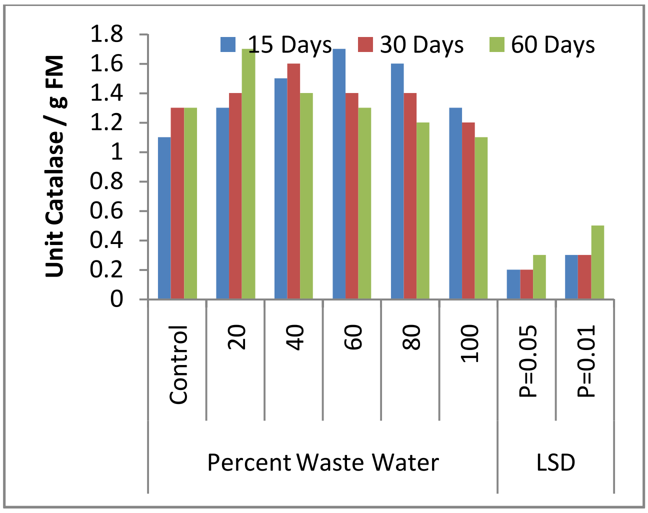


Fig 5: Effect of different conc. of waste water on Catalase

DISCUSSION

Maximum fresh matter yield was found at 40 % level of waste water at 15, 30 and 60 days. But recent studies evaluating *Ceratophyllum demersum* or CWM as a dietary ingredient for aquatic species are not readily available, and thus difficult to compare with the findings of this study. Hajra [16] reported that the submerged aquatic weed *Ceratophyllum demersum* offered freshly and supported the growth even though the fiber content of CWM is moderately low, its fiber type might be the indigestible derivatives such as lignin and cellulose which are commonly identified in other aquatic weeds including *Pistia stratiotes* and *Lemna perpusilla* [17].

Maximum dry matter yield were recorded at 40% level of waste water in 15, 30 and 60 days growth. The results are in support with Patel and Kanungo [18] who studied increase in biomass production of *Ceratophyllum demersum*, L. Shakya et al [29] studied with different level of waste water for 60 days and found increased in 25% levels of effluents.

Maximum chlorophyll content in 15 days growth at 40 % level and at 20% level of 30 and 60 days growth of *Ceratophyllum demersum*was observed. Singh and Singh [20] reported increase in chlorophyll content of *Lemna minor* at primary concentration, and then significantly decrease in industrial and sewage water.

Catalase activity was found to be maximum at 60% level of waste water in 15 days growth of*Ceratophyllum demersum* plant, 30 days growth at 40% and at 20% level of waste water in 60 days growth. Heavy metal-induced H_2O_2 accumulation is prevented by catalase and peroxidases. Hydrogen peroxide is a very toxic reactive oxygen species [21]. The increased activity of CAT indicates effective scavenging of H2O2. However, in this study, activities of CAT once inceased then gradually decreased. The reason for this decrease may be due to increased activities of peroxidases which are also detoxifiers of H2O2. Additionally, Piotrowska et al [22] reported that inhibition of CAT activity was probably caused by the toxic effect of H2O2 and other ROS. Similar to results of this study, reduced activity of CAT was reported in plants under Cd (*Bacoppa monnieri*, *W. arrhiza* and *G. densa*), Cu (*L. minor*, *S. polyrhiza*), Pb (*W. arrhiza*) and Cr (cauliflower) stresses [23-26].

Maximum ascorbic acid content was recorded at 40% level of waste water in 30 days growth, while at 20% level of waste water in 60 days growth. However 60% level of waste water showed maximum ascorbic acid content of 15 days growth. Ascorbic acid, a natural antioxidant scavenges free radicals generated by heavy metals [27]. Ascorbic acid content in plant and metal concentration was positively and significantly correlated with Cd, Pb, Zn and Ni concentrations. Sinha et al [28] have also reported higher production of ascorbic acid in fenugreek plants grown in soil amended with tannery sludge to nullify the adverse effects of heavy metals. *Colocasia esculentum* and *Raphanus sativus* grown in wastewater irrigated area of Durgapur, West Bengal showed higher production of ascorbic acid under wastewater irrigation [29].

CONCLUSION

It were concluded that aquatic plants were beneficial as bioremediation treatment of municipal waste water before using for gardening purpose or irrigation of crop plants. It is safe to use at least 50% dilution of municipal waste water for bioremediation treatment for beneficial results. This study leads an option to test some other aquatic plants for bioremediation treatment of municipal waste water and also for treatment of othr industrial effuluents.

CONFLICT OF INTEREST

There is no conflict of interest between the authors.

ACKNOWLEDGMENT

Authors are thankful to Department of Botany, Bundelkhand university, Jhansi.

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CITATION OF THIS ARTICLE

Amit Pandey and R. K. Verma. Assessment of the Efficiency of *Ceratophyllum demersum* in Waste Water Treatment.Bull. Env.Pharmacol. Life Sci., Vol 9[4] March 2020 : 117-123