



Morphological Evaluation of Freshwater Ornamental Aquatic Plant Waterwort, *Elatine gratioloides* A. Cunn under Different Organic Substrate

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SOK and VIK conceived and designed the experiment, wrote the protocol and first draft of the manuscript. Author SOK performed the statistical analysis, Author SOK, AP and SS managed the literature searches and the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

An investigation was carried out to evaluate the efficacy of different organic substrate on morphological traits of the freshwater ornamental aquatic plant, waterwort *Elatine gratioloides* in the mud pots of 3.5 L capacity at College of Fisheries, GADVASU, Ludhiana, Punjab for 5 months duration. An experiment was planned in completely randomized design with five treatments viz., control- without manure (T₀), Vermi compost (T₁), poultry manure (T₂), cattle manure (T₃) and goat manure (T₄) in triplicate to study the effect of organic substrate on growth and development of *E. gratioloides*. The study revealed that, increase in plant biomass, total plant length and number of leaf and runners was observed in treatments containing high nitrogen content. Comparatively, poultry manure (T₂) illustrated superior results and showed significant difference among the treatments, except for the cattle manure (T₃). Since cattle manure is easily available in local market

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at an affordable price; we recommend that its application @ 2% with soil/sand mixture (2:1) helps to increase plant biomass, total plant length and produced good leaf quality for culture and propagation of ornamental aquatic plant waterwort.

Keywords: Aquatic plant; cattle manure; morphological; waterwort; organic substrate; aquarium plant.

1. INTRODUCTION

Aquatic plants refer to any organisms living in water that photosynthesize and complete part or all of their life cycle in or near the water [1]. Aquatic plants are ecologically important in the system because they provide habitat for many benthic and pelagic organisms. They also help in stabilizing sediment, improving water chemical nutrients from water and sediment into plant matter through photosynthesis, which serves as a food for the animals. Further, they act as a buffer strip, absorbing many harmful pollutants from water [2].

Ornamental aquatic plant exportation began in Brazil in the 1930s [3,4]. Aquarium plants industry in domestic and international market has developed rapidly, and artificial hybrids have already replaced many natural species in markets [5]. In India, aquarium plant cultivation is an upcoming enterprise, which has not progressed to the level of its counterpart i.e. The ornamental fish trade [6]. The cultivation might help to reduce paucity of aquarium plants in domestic market, as it is mainly dependent upon wild collection and at the same time it may help to conserve the biodiversity. To satisfy the need of hobbyists and traders, it is necessary to expand this occupation into small scale industry by setting up aquatic plant nurseries in different parts of country. Therefore, for the sustainable development of the industry, it is necessary to strengthen the culture and propagation techniques for commercially important species.

Waterwort *Elatine gratioloides* (Family: Elatinaceae) belonging to Order Herbaceous is an attractive sprawling and upright growing aquatic plant which has bright green leaves on brittle stems. It is widely used in aquascaping industry for arranging aquatic plants with rocks, stones and driftwood, as it adds aesthetic value to the aquarium. Propagating aquatic plants is both science and an art; for successful propagation it requires the apt knowledge regarding the requirement of organic and inorganic fertilizer, which is provided as substrate. In natural environment, nutrient supply in form of macronutrient, as well as micronutrient, plays an important role in determining plant

community structure [7]. The fertilizer derived from animals, plants and microorganisms are usually called organic manures or farmyard manure. Many varieties of farmyard manure are locally produced based on available natural resources and are commercially available in local market at cheap price. It can be utilized to study the plant growth under controlled condition, which in turn will help for the mass cultivation of ornamental aquatic plants. Ornamental aquatic plants are grown in pots to control the spreading of plant and leaching of fertilizers from the growing medium within the pond [8,9]. Keeping above mention points into consideration, the present study was planned to examine the efficacy of different farmyard manures for culture and propagation of ornamental aquatic plant waterwort, *E. gratioloides*.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was conducted to evaluate the effect of farmyard manures on the growth of *E. gratioloides* in the mud pots of 350 ml capacity at College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana for five months. Alluvial soil available in college campus was used for the experiment.

2.2 Experimental Plant

Freshwater ornamental aquatic plant *E. gratioloides* was obtained from a domestic ornamental fish market in Kurla, Mumbai, Maharashtra. The identification of plant was carried out by using the distinguishing characters [10,11]. Then it was planted in the pots for future growth and was placed in the fibre-reinforced plastic (FRP) tanks. At the beginning of trial, a horizontal branch arising from the base of a plant consisting of buds or nodes at its tip (produces new plant called as runner) was cut with scissors and used as plantlet.

2.3 Experimental Design

Five treatments such as control- without manure (T_0), Vermi compost (T_1), poultry manure (T_2),

Table 1. Chemical composition of the growing media (soil, sand and manures)

Parameter	Soil	Sand	Vermi compost	Poultry manure	Cattle manure	Goat manure
pH	7.69	8.93	6.69	6.80	6.86	8.56
Electric conductivity (ds/m)	0.151	0.133	1.02	3.76	0.717	0.324
Organic Carbon (%)	3.1	2.8	5.5	9.4	7.1	9.0
Available Nitrogen (%)	0.07	0.02	0.49	1.26	0.83	0.79
Available Phosphorous (%)	0.035	0.037	0.241	3.945	0.522	0.401
Available Potassium (%)	0.14	0.09	0.18	0.65	0.24	1.14
Zinc (ppm)	42.3	43.2	52.8	148.8	96.9	54.9
Iron (ppm)	2296	2171.5	2215.5	1769.4	1330.2	1782.5
Manganese (ppm)	13.5	17.7	42.3	137.1	47.1	24.9
Copper (ppm)	9.6	10	11.45	20.65	13.3	11.95

cattle manure (T_3) and goat manure (T_4) were used as an organic substrate for growing *E. gratioloides* in triplicate (Table 1). The mud pots of 3.5 L capacity were filled with a mixture of 1 kg of soil and sand (2:1) supplemented with 2% of the organic substrate which were thoroughly mixed and then remaining space was filled by 1 kg sand and top-up with small stones (pebbles) to avoid the loss of media. Chemical composition of growing media (manures, soil and sand) like pH, conductivity, organic carbon (OC), macronutrients: available nitrogen (N), phosphorous (P) and potassium (K) and micronutrients: zinc (Zn), iron (Fe), manganese (Mn) and Copper (Cu) etc. were determined as per the standard methods [12].

2.5 Morphological Studies

Morphological trait is a characteristic of a living thing that we can observe, such as size, color, shape, capabilities, behaviors, etc. In genetics, you can divide all traits into two categories based on their effects on an organism's phenotype: qualitative and quantitative. For qualitative traits the plants were examined visually for differences in nature of branch and leaves, color of leaves, stem, and shape of leaves, while quantitative traits vegetative characters such as root length (RL), shoot length (SL), root to shoot length ratio (RL/SL), plant length (PL), leaf length (LL), leaf width (LW). Leaf length/leaf width (LL/LW) ratios were studied.

2.6 Water Quality Parameters

Water samples were collected for physicochemical analyses like temperature ($^{\circ}\text{C}$), pH, specific conductivity (μScm^{-1}), dissolved oxygen (mgL^{-1}), Carbon dioxide (mgL^{-1}), total

alkalinity (mgL^{-1}), total hardness (mgL^{-1}), ammonia (mgL^{-1}), nitrate (mgL^{-1}), nitrite (mgL^{-1}) were determined as per the standard methods [13].

2.7 Statistical Analysis and Interpretation of Data

Statistical analysis of the data was performed with a statistical package (SPSS 16.0, SPSS Inc., Richmond, CA, USA). Values was presented as means \pm standard error of the mean. Data for the growth parameters were tested for homogeneity of variances, and then possible differences were tested using one-way ANOVA for morphological parameters and followed by a Duncan's multiple comparison to find out the difference between treatments.

3. RESULTS AND DISCUSSION

3.1 Qualitative Analysis

In present study all the treatments showed erect branching habit with bright green coloured shoots (Table 2). The green colouration in stem was possibly due to chlorophyll pigment concentration in all treatments except T_1 and control, while leaves were bright green in colour, elongated, and oblong in shape [14]. The above discrepancy in qualitative traits may possibly have occurred due to the genetic makeup of these plants, as they were kept under homogenous environmental conditions.

3.2 Quantitative Analysis

ANOVA results shows that, at the initiation of trail there was no significant differences ($p>0.05$) in the plant biomass and total length among all

treatments. The organic substrate had significant effects ($p < 0.05$) on the plant length and number of leaves in Waterwort, *E. gratioloides* (Table 3). The plant raised with poultry manure as substrate recorded maximum total length, number of leaves as well as number of runners. Moreover, a significant difference was observed in number of runners produce by plant during the trail and it was supported by the data, which showed higher growth rate in all treatments except T_1 and T_0 (control). No significant difference was observed between plant biomass in terms of grams in T_3 (135.67 ± 7.62) and T_2 (131.67 ± 7.22) treatments, while other treatments were significantly affected by use of organic substrate and lowest plant biomass was recorded in control (77.23 ± 4.01). According to previous studies, use of organic manure as substrate helps to increase the plant biomass, plant length and produced good leaf quality [2,15,16]. The results from the above studies were in the line with the present study, as the growth of waterwort was recorded less in vermi compost due to its higher organic carbon content than essential available nitrogen. It was observed that maximum biomass, number of leaf and runners were in the T_2 and T_3 treatment which contained high available nitrogen. No mortality or plant deformity was observed in any treatments during the experiment. Plant roots absorb water and nutrients from the surrounding soil, and a healthy root system is key to a healthy plant. The root: shoot ratio is one measure to assess the overall health of your plants [17]. The maximum root: shoot ratio was found in the T_2 compare to other treatments. The decrease in root-to-shoot ratio might have arisen due to the increased growth of shoot in growth studies. Leaf length to leaf width ratio is a measure of the efficiency with which a plant deploys its photosynthetic resources [18]. In present study, when analyzing the leaf length to leaf

width ratio it was significantly affected by use of different substrate for growth of waterwort and also due to paucity of photosynthetic resources.

3.3 Water Quality Analyses

In the present study, the temperature fluctuation was recorded between 28 to 32°C, while pH was about 7.5 (Table 4). Specific conductivity of water showed gradual decline after first month in all the treatments, except in control. Owing to photosynthesis, dissolved oxygen concentration was found to be increased in all the treatments and there was no significant difference among the treatments ($p > 0.05$). Most of the macrophytes supplement free dissolved CO_2 with bicarbonate [19] however, they are more readily able to assimilate dissolved CO_2 . During present study, free carbon dioxide was absent in all the treatments up to the end of the experiment. Water hardness and alkalinity ranged from 212.24-234.14 mgL^{-1} and 232.40-268.19 mgL^{-1} respectively in all the treatments including control and highest value was observed in poultry manure. According to the result of our investigation, the estimated values of hardness and alkalinity were found within the range. The optimum values of hardness ($CaCO_3$) and alkalinity for aquaculture ranges from 63 to 250 mgL^{-1} [20] and 50 to 300 mgL^{-1} [21] respectively. In the present study ammonia-nitrogen ranged from 0.09 to 0.03 mgL^{-1} , nitrate-nitrogen was less than 0.04 mgL^{-1} and nitrite-nitrogen was around 0.01-0.02 mgL^{-1} . Values were found to significantly higher in T_3 treatment (poultry manure) compared to other treatments ($p < 0.05$), as it is a well-known fact that aquatic plant reduces ammonia, nitrate and nitrite by direct absorption, while the absorption capacity differs as per different manures [22] and [23].

Table 2. Qualitative traits of waterwort during experiment

Treatments	Qualitative traits				
	Nature of the branch	Colour of the stem	Colour of the leaves	Shape of the leaves	Nature of leaves
Control (T_0)	e	lg	bg	ob	el
T_1	e	lg	bg	ob	el
T_2	e	g	bg	ob	el
T_3	e	g	bg	ob	el
T_4	e	g	bg	ob	el

Note: e-erect, lg-light green, g-green, bg-bright green, dg-dark green, ob-oblong. el-elongated

Table 3. Quantitative traits of waterwort during experiment

Treatments	Growth parameters									
	Shoot length	Root length	Total plant length	RL/SL*	No. of runners	No. of leaves	Leaf height	Leaf width	Biomass	LL/LW**
T ₀	65.67±2.31 ^c	28.46±1.86 ^a	93.13±3.97 ^c	0.45±0.03 ^b	5.00±0.57 ^b	137.33±12.73 ^c	15.43±0.75 ^b	1.66±0.14 ^b	77.23±4.01 ^d	8.29±0.05 ^{bc}
T ₁	68.23±5.34 ^{bc}	30.70±5.03 ^a	98.93±7.19 ^b	0.40±0.01 ^c	4.67±0.67 ^b	148.67±14.33 ^c	14.97±0.83 ^c	1.70±0.15 ^b	88.67±4.06 ^c	8.94±0.06 ^a
T ₂	77.00±5.86 ^a	30.50±1.26 ^a	107.50±5.92 ^a	0.50±0.02 ^a	7.67±0.33 ^a	205.67±08.99 ^a	18.23±0.83 ^a	2.05±0.07 ^a	131.67±7.22 ^a	8.15±0.15 ^c
T ₃	71.33±1.76 ^b	30.17±2.45 ^a	101.50±3.07 ^{ab}	0.45±0.03 ^b	8.00±1.15 ^a	186.67±11.62 ^b	17.03±0.86 ^a	1.70±0.12 ^b	135.67±7.62 ^a	8.03±0.08 ^c
T ₄	65.80±3.06 ^c	29.43±0.74 ^a	95.23±2.62 ^b	0.44±0.03 ^b	8.00±1.53 ^a	199.33±08.41 ^a	16.93±0.50 ^{ab}	2.10±0.05 ^a	104.33±9.91 ^b	8.39±10 ^b

Note: Different superscripts in the same column mean significant difference between treatments (Duncan's multiple comparisons, $P < 0.05$). *Root to shoot length ratio; ** Leaf length to leaf weight ratio

Table 4. Water quality parameters in different treatments during experiment

Parameter	Treatments				
	Control (T ₀)	T ₁	T ₂	T ₃	T ₄
Temperature (°C)	30.93 ^a ±0.31	30.92 ^a ±0.35	30.94 ^a ±0.25	30.88 ^a ±0.27	30.93 ^a ±0.31
pH	7.54 ^{ab} ±0.03	7.51 ^b ±0.03	7.46 ^c ±0.02	7.57 ^a ±0.03	7.51 ^b ±0.03
Specific conductivity (µS _{cm} ⁻¹)	1.08 ^{ab} ± 0.03	1.04 ^b ± 0.03	1.12 ^a ± 0.04	1.11 ^a ± 0.04	1.03 ^b ± 0.01
Dissolved oxygen(mgL ⁻¹)	6.16 ^a ± 0.24	6.21 ^a ± 0.37	6.28 ^a ± 0.31	6.36 ^a ± 0.23	6.32 ^a ± 0.42
Free carbon dioxide (mgL ⁻¹)	Nil	Nil	Nil	Nil	Nil
Total hardness (mgL ⁻¹)	202.24 ^c ±3.57	204.12 ^c ±2.63	222.43 ^a ±2.31	224.14 ^a ±1.71	211.76 ^b ±2.10
Total alkalinity (mgL ⁻¹)	232.40 ^c ±0.94	245.57 ^b ±1.13	234.14 ^c ±0.92	238.94 ^b ±1.44	268.19 ^a ±1.12
Ammonia-nitrogen (mgL ⁻¹)	0.03 ^c ± 0.01	0.05 ^b ± 0.01	0.09 ^a ± 0.02	0.05 ^b ± 0.01	0.07 ^{ab} ± 0.02
Nitrate-nitrogen (mgL ⁻¹)	0.02 ^b ± 0.01	0.02 ^b ± 0.01	0.04 ^a ± 0.01	0.02 ^b ± 0.01	0.03 ^{ab} ± 0.01
Nitrite-nitrogen (mgL ⁻¹)	0.01 ^b ± 0.01	0.01 ^b ± 0.01	0.02 ^a ± 0.01	0.01 ^b ± 0.01	0.01 ^b ± 0.01

Note: Different superscripts in the same column mean significant difference between treatments (Duncan's multiple comparisons, $P < 0.05$).

4. CONCLUSION

This study illustrated new and important insights into potential application of organic manure especially cattle and poultry manure as a substrate for culture and propagation of aquatic plants, which in turn will help for the expansion of ornamental aquatic plant industry from grass root level. Both cattle and poultry manure showed better results during morphological evaluation of *E. gratioloides*. Though the treatments comprising growth medium as poultry manure had slightly better results compared to the one with cattle manure which is easily available at cheaper price in local market and contains less amount of nitrogen. In conclusion we suggest that cattle manure @ 2% with soil/sand mixture (2:1) helps to increase plant biomass, total length and produced good leaf quality of waterwort under simulated conditions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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