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## Quantitative and qualitative differences in morphological traits of freshwater ornamental aquatic plant water wisteria, *Hygrophila difformis* under different organic substrate

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### Abstract

The field study was conducted to investigate the quantitative and qualitative differences in morphological traits of freshwater ornamental aquatic plant Water wisteria, *Hygrophila difformis* under different organic substrate in mud pots of 3.5 liter capacity for 5 months under controlled environmental conditions. Results showed that maximum biomass, plant length, number of leaf and runners were observed in the treatments which contain high available nitrogen. Further during the study it was confirmed that water wisteria is a good model to study heterophylly, as leaves in all treatments from pinnately veined shape changed to pinnately lobed in nature. No mortality or plant deformity was observed in any treatments during the experiment. It can be concluded that the application of cattle manure at the rate of 2% with soil/sand (2:1) mixture is good for culture and propagation of ornamental aquatic plant, water wisteria as it is easily available at cheaper price in local market compare to other manures.

**Keywords:** aquatic plant, morphological, water wisteria, organic substrate, heterophylly

### 1. Introduction

Aquatic plants are ornamental species: they are planted in aquaria for their beauty, but also to maintain water quality. They are selected for shape, plant colour and size [19]. Like any other plant, aquatic plants also fix inorganic carbon (CO<sub>2</sub>) into organic carbon through photosynthesis and release oxygen into the water medium, which is important for fish and other aquatic organisms that depend on dissolved oxygen to survive. They absorb nutrients, resulting in their less availability for algae, hence making algal blooms less likely. In addition, aquatic plants provide food, shelter and breeding places to fishes being reared together with plants [13]. Export of ornamental aquatic plants began in 1930s in Brazil [7]. It has been one source of secondary income beside ornamental fish trade for aquarium companies [10]. 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 [20]. The supply is mainly dependent upon wild collection and may lead to ecological imbalance due to overexploitation along with non-availability of desirable species. To develop this activity into small scale business, there is need to set up an ornamental aquatic plant nursery for successful culture and propagation of aquatic plants. This in turn will help to cater the needs of hobbyists and traders in India. Hence, for the sustainable growth of the trade, it is necessary to intensify the culture and propagation techniques for commercially important species. Fast growing semi-aquatic plant water wisteria (*Hygrophila difformis*) shows wide variation in leaf shapes as it grows older, as the transformation of simple leaves happens into highly branched compound leaves [11] and adds much more aesthetic value to the aquarium outlook. In many natural environments, nutrient supply is also an important factor in determining plant community structure [5]. The natural source of nutrients derived from animals, plants and microorganisms are usually called organic manure viz., poultry manure, goat manure, cattle manure and vermi compost. For successful propagation and better growth of aquarium plants, there rises a need to understand different organic manure requirement which are used as substrate. Organic manures can be utilized as the substrate to study the plant growth under control condition, which in turn will help for mass cultivation of ornamental aquatic plants. In this paper we present a study on the qualitative and quantitative differences in morphological traits of freshwater ornamental plant water wisteria, *H. difformis* under different organic substrates. The overall objective of our

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study was to investigate the efficacy of different organic manures for culture and propagation of ornamental aquatic plant *water wisteria*.

## 2. Materials and Methods

The experiment was conducted at College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana for five months. Freshwater ornamental aquatic plant *water wisteria*, *H. difformis* was obtained from domestic ornamental fish market in Kurla, Mumbai, Maharashtra. The identification of plant was carried out by using the distinguishing characters [6, 17]. Five treatments such as control (without application of manure) ( $T_0$ ), vermi compost ( $T_1$ ), poultry manure ( $T_2$ ), cattle manure ( $T_3$ ) and goat manure ( $T_4$ )

were used as organic substrate for growing *water wisteria* in triplicate. Alluvial soil available in college campus was used for the experiment. Chemical composition of growing media (manures, soil and sand) was determined as per the standard methods as given in Table 1 [1]. The mud pots of 3.5 liter capacity were filled with 1 kg of soil and sand (2:1) supplemented with 2% different organic manures as per experiment design which was 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. Then a horizontal branch arising from the base of a plant that consisting buds or nodes at its tip that produces new plants called as runners was cut with scissor and planted in the pots for future growth and was placed in the plastic pool.

**Table 1:** Chemical composition of different organic manures, soil and sand

	pH	EC (ds/m)	Organic Carbon (%)	Available N (%)	Available P (%)	Available K (%)
Vermi compost	6.69	1.02	5.5	0.49	0.241	0.18
Poultry manure	6.80	3.76	9.4	1.26	3.945	0.65
Cattle manure	6.86	0.717	7.1	0.83	0.522	0.24
Goat manure	8.56	0.324	9.0	0.79	0.401	1.14
Soil	7.69	0.151	3.1	0.07	0.035	0.14
Sand	8.93	0.133	2.8	0.02	0.037	0.09

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 for quantitative traits vegetative characters such as shoot length (SL), root length (RL), Shoot length/root length ratio, plant length (PL), leaf length (LL), leaf width (LW), Leaf length/leaf width (LL/LW) ratio were recorded. Water samples were collected for physicochemical analysis like temperature ( $^{\circ}\text{C}$ ), pH, specific conductivity ( $\mu\text{S}/\text{cm}$ ), dissolved oxygen (mg/l), Carbon dioxide (mg/l), total alkalinity (mg/l), total hardness (mg/l), ammonia (mg/l), nitrate (mg/l), nitrite (mg/l) were determined as per the standard methods [2].

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 phenotypic parameters and followed by a Duncan's multiple comparison to find out the difference between treatments.

## 3. Results and Discussions

The occurrence of heterophylly indicates transformation in prominent phenotypic character, as leaves changes their morphology in response to favorable environmental conditions [3, 21]. Heterophylly is observed in a wide range of vascular plants including both dicots and monocots [4, 16]. There are few aquatic plants, which exhibit heterophylly viz., *Anubias*, *Limnophila*, *Callitriche*, *Hygrophilia* etc. *H. difformis* (Acanthaceae), is one of the fast growing semi-aquatic plant that exhibits a variety of leaf shapes, from simple leaves to highly branched compound leaves, depending on the environment [11]. Heterophylly may increase the fitness of aquatic plants by decreasing leaf damage from mechanical forces or herbivores, by decreasing water loss or by enhancing photosynthesis [15]. In present study all the treatments showed semi-erect branching habit with light green coloured shoots (Table 2). The light green colouration in stem was possibly due to chlorophyll pigment concentration compare to other treatments and control; while leaves were spring green [22] in all treatments with pinnately veined shape and pinnately lobed nature [15]. This variation in qualitative characters may be due to the genetic makeup of these plants as they were maintained in similar environmental and ecological conditions. As expected, *H. difformis* showed heterophylly character which is the similar findings of earlier study [16].

**Table 2:** Qualitative traits of water wisteria 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 ( $T_0$ )	se	lg	g	pv	pl
$T_1$	se	lg	g	pv	pl
$T_2$	se	g	g	pv	pl
$T_3$	se	lg	g	pv	pl
$T_4$	se	lg	g	pv	pl

Note: Se-sub erect, lg-light green, g-green, dg-dark green, pv-pinnately veined, pl-pinnately lobed

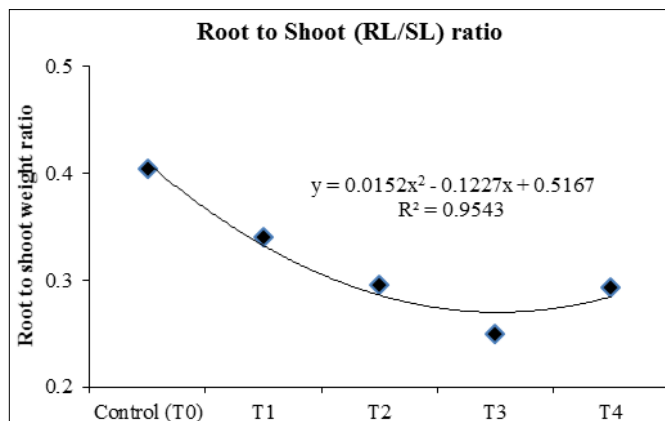
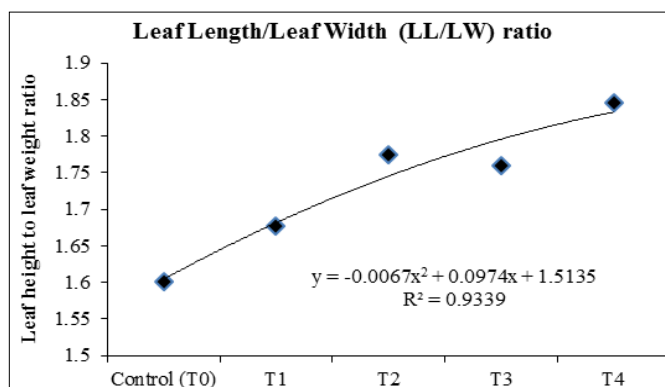
**Table 3:** Quantitative traits (Growth parameters) of water wisteria during experiment

Treatments	Growth Parameters							
	SL	RL	PL	Runners	No. of Leaves	LH	LW	Biomass
Control(T <sub>0</sub> )	42.66±4.91 <sup>c</sup>	17.23±2.03 <sup>b</sup>	59.90±6.83 <sup>c</sup>	6.67±0.88 <sup>b</sup>	163.33±14.62 <sup>c</sup>	5.84±0.20 <sup>b</sup>	3.64±0.11 <sup>b</sup>	102.67±5.81 <sup>b</sup>
T <sub>1</sub>	36.00±5.57 <sup>c</sup>	12.23±1.63 <sup>c</sup>	48.23±7.19 <sup>d</sup>	2.33±0.33 <sup>c</sup>	71.66±7.36 <sup>d</sup>	5.64±0.77 <sup>b</sup>	3.36±0.29 <sup>c</sup>	86.00±5.29 <sup>c</sup>
T <sub>2</sub>	70.33±6.06 <sup>ab</sup>	20.73±1.46 <sup>a</sup>	91.06±7.51 <sup>a</sup>	9.66±1.76 <sup>a</sup>	236.66±10.37 <sup>b</sup>	7.58±0.27 <sup>a</sup>	4.27±0.24 <sup>a</sup>	174.66±12.86 <sup>a</sup>
T <sub>3</sub>	73.33±3.33 <sup>a</sup>	18.33±0.88 <sup>ab</sup>	91.66±3.28 <sup>a</sup>	7.00±2.52 <sup>ab</sup>	328.33±16.18 <sup>a</sup>	7.46±0.43 <sup>a</sup>	4.24±0.23 <sup>a</sup>	180.00±10.26 <sup>a</sup>
T <sub>4</sub>	61.67±6.01 <sup>b</sup>	18.03±2.87 <sup>ab</sup>	79.70±7.69 <sup>b</sup>	8.00±0.58 <sup>ab</sup>	264.33±26.86 <sup>b</sup>	6.96±0.50 <sup>a</sup>	3.77±0.45 <sup>ab</sup>	112.66±7.69 <sup>b</sup>

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

The ANOVA results indicated that, there were no significant differences in the initial plant length and biomass among all treatments ( $p > 0.05$ ). The organic manures had significant effects on the plant length and number of leaf of Water wisteria, *H. difformis* ( $p < 0.01$ ) (Table 3). Maximum plant length, number of leaves was  $91.66 \pm 3.28$  and  $328.33 \pm 16.18$  respectively observed in plant grown with cattle manure. Further there were no significant differences 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 vermi compost. Plant biomass was recorded as  $102.67 \pm 5.81$ ,  $86.00 \pm 5.29$ ,  $174.66 \pm 12.86$ ,  $180.20 \pm 10.26$  and  $112.66 \pm 7.69$  gm in control, vermi compost, poultry compost, cow dung and goat dung respectively. There were few studies conducted on application of organic manure in order to increase the plant height biomass, plant length and produced good leaf quality [8, 12, 18]. The results from these studies were in the line with the present study, as the growth of water wisteria was recorded less in vermi compost due to its higher organic carbon content than essential available nitrogen. It was observed that maximum biomass, plant length, number of leaf and runners were observed in the treatments which contain high available nitrogen.

Healthy root system is the most essential key for all healthy plants as it allows the absorption of water and nutrients from the surrounding soil and root: shoot ratio is also a main component for assessing of the overall healthy condition of targeted plant [9]. It was seen that T<sub>3</sub> (cattle manure) compromised the root to shoot wet weight ratio (Figure 1). The maximum value was found in the control 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 [14]. In present study, when analyzing the leaf length to leaf width ratio for different treatments, a lower value was seen in control and T<sub>1</sub> while higher values were found in T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> (Figure 2).

**Fig 1:** Root to shoot weight ratio during experiment on *H. difformis***Fig 2:** Leaf length to leaf width ratio during experiment on *H. difformis*

During the course of the experiment, the temperature ranged from 28 to 32 °C, pH was around 7.5 and ammonia nitrogen was less than 0.2 mg/L (Table 4). In the present study, dissolved oxygen increased in all the treatments including control due to photosynthesis and there was no significant difference among the treatments ( $p > 0.05$ ), while carbon dioxide was found to be absent. No mortality or plant deformity was observed in any treatments during the experiment ( $p > 0.05$ ).

**Table 4:** Water quality parameters in different treatments during experiment period

Parameter	Treatments				
	Control (T <sub>0</sub> )	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
Temp (°C)	30.77 <sup>a</sup> ±0.36	30.78 <sup>a</sup> ±0.32	30.69 <sup>a</sup> ±0.37	30.63 <sup>a</sup> ±0.40	30.76 <sup>a</sup> ±0.36
pH	7.52 <sup>b</sup> ±0.03	7.41 <sup>c</sup> ±0.01	7.49 <sup>b</sup> ±0.02	7.61 <sup>a</sup> ±0.03	7.43 <sup>c</sup> ±0.02
Specific conductivity (µS/cm)	1.07 <sup>ab</sup> ± 0.03	1.04 <sup>b</sup> ± 0.03	1.10 <sup>a</sup> ± 0.02	1.12 <sup>a</sup> ± 0.04	0.99 <sup>c</sup> ± 0.01
Dissolved oxygen(mg/L)	6.12 <sup>a</sup> ± 0.35	6.15 <sup>a</sup> ± 0.62	6.18 <sup>a</sup> ± 0.48	6.26 <sup>a</sup> ± 0.59	6.22 <sup>a</sup> ± 0.84
Free Carbon dioxide (mg/)	Nil	Nil	Nil	Nil	Nil
Total hardness (mg/L)	213.57 <sup>c</sup> ±2.66	215.76 <sup>c</sup> ±2.37	233.33 <sup>a</sup> ±1.50	235.00 <sup>a</sup> ±1.71	221.76 <sup>b</sup> ±2.10
Total alkalinity (mg/L)	232.40 <sup>c</sup> ±0.94	245.57 <sup>b</sup> ±1.13	234.14 <sup>c</sup> ±0.92	238.94 <sup>b</sup> ±1.44	268.19 <sup>a</sup> ±1.12
Ammonia-nitrogen (mg/L)	0.04 <sup>c</sup> ± 0.01	0.06 <sup>b</sup> ± 0.02	0.11 <sup>a</sup> ± 0.05	0.06 <sup>b</sup> ± 0.03	0.08 <sup>ab</sup> ± 0.04
Nitrate-nitrogen (mg/L)	0.02 <sup>b</sup> ± 0.01	0.02 <sup>b</sup> ± 0.01	0.04 <sup>a</sup> ± 0.01	0.02 <sup>b</sup> ± 0.01	0.03 <sup>ab</sup> ± 0.01
Nitrite-nitrogen (mg/L)	0.01 <sup>b</sup> ± 0.01	0.01 <sup>b</sup> ± 0.01	0.02 <sup>a</sup> ± 0.01	0.01 <sup>b</sup> ± 0.01	0.01 <sup>b</sup> ± 0.01

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

#### 4. Conclusions

In the present study, the application of cattle manure and poultry manure illustrated better results during morphological evaluation of qualitative and quantitative traits of freshwater ornamental aquatic plant water wisteria. Comparatively, cattle manure is easily available in local market at cheaper price and also contains less amount of nitrogen. In conclusion we suggest that cattle manure @ 2% with soil/sand mixture (2:1) helps to increase plant length, biomass, and produced good leaf quality of water wisteria under controlled conditions.

#### 5. Acknowledgement

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