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Competition between of invasive *Hydrilla verticillata* L.f. and native *Ceratophyllum demersum* L. in monocultures and mixed cultures experimental

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Abstract

The present study aims to assess inter-specific competition between *Hydrilla verticillata* and *Ceratophyllum demersum*. A competition experiment design has five treatment combinations to terminal shoots of *C. demersum* only, terminal shoots of *H. verticillata* only and three different treatment combinations of *C. demersum* and *H. verticillata* together. Our results showed parameters growth of *C. demersum* were decreased of which, total chlorophyll, protein, and biomass while Superoxide dismutase (SOD), and Catalase (CAT) enzymes increase were significantly (p<0.05) compare with control treatment. While *H. verticillata* were increased total chlorophyll, biomass, CAT and little increase in protein and SOD were significantly (p<0.05) compare with control treatment. Therefore, due to the competition, with the introduction of *C. demersum*, the performances growth of *H. verticillata* were increased. Based on our result, showed *H. verticillata* and *C. demersum* species were coexist, *C. demersum* will have a competitive advantage over *H. verticillata*. Therefore, this study suggests, *H. verticillata* could out-compete for *C. demersum* in many situations, that despite the similar ecology.

Keywords: Aquatic plants, Ceratophyllum, Hydrilla, Invasive, Native.

التنافس بين النبات الغازي Hydrilla verticillata والنبات المحلي Ceratophyllum في مزارع تجريبية مفردة ومختلطة

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الخلاصة

تهدف الدراسة الحالية الى تقييم التنافس بين نباتي الهايدرلا Hydrilla verticillata والشمبلان Ceratophyllum demersum . صممت تجربة التنافس على خمسة معاملات من البراعم الطرفية للمجموع الخضري لنبات الهايدرلا فقط ونبات الشمبلان فقط وثلاث تداخلات مختلفة للنباتين معاً. أظهرت النتائج انخفاض معايير النمو لنبات الشمبلان حيث اختزلت قيم للكلوروفيل الكلي، البروتين، والكتلة الحية بينما ارتفاع قيم انزيمي الكتاليز (CAT)والسوبر اوكسيد دسميوتيز (SOD) فقد سجلت فروق معنوية (P<0,05)

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بمعامل السيطرة. بينما سجلت الهايدرلا زيادة في قيم الكلوروفيل الكلي، والكتلة الحية وانزيم الكتاليز وزيادة ضئيلة في قيم البروتين وانزيم السوبر اوكسيد دسميوتيز فقد سجلت فروق معنوية (p<0,05) مقارنة بمعامل السيطرة. واستنادا لهذه الدراسة فان التداخل النتافسي بين النباتين يشير الى وجود ميزة تنافسية عندما يتعايش النباتين سوياً، فقد اظهرت النتائج ان هنالك تفوق للهايدرلا على الشمبلان في العديد من الحالات في ظروف يبنية متماتلة.

1. Introduction:

Nitrogen (N) and phosphor (P) is one of the most limiting plant nutrients affecting the growth, nutrient uptakes of aquatic plants, despite phosphor being found in a small amount in the water. [1,2]. The ecosystem structure, processes, and function depended on Nitrogen and Phosphorus that are considered of important nutrients for since their availability limits the production of plant biomass and growth [3]. For example, the combined application of N and P increases root surface area, root length, and root-shoot mass [4]. As well as for the storage, acquisition, and use of energy [5].

Previous studies have shown that N supplementations could significantly affect shoot morphology and nutritional status of nursery seedlings [6]. The growth promoting effect of N (up to the optimum level) increases cytokinin production, which subsequently affects cell wall elasticity [7], the number of meristematic cells, and cell growth [8]. In addition, N fertilization also increases seedling height and root collar diameter [9].

Macrophytes play a crucial role in the dynamics of aquatic ecosystems, as they are the main primary producers and also the base diversified plants, which negatively impact water bodies [10]. Submersed macrophytes play a key role in freshwater shallow ecosystems; they provide habitats and refuges for predatory fish and zooplankton, which may indirectly inhibit phytoplankton abundance [11].

Hydrilla verticillata is diagnosed as an invasive species in Iraq. It could be introduced to the country via the ornamental aquatic plant industry in the early 1980s. On the other hand, *Ceratophyllum demersum* considered a native in many areas of the world.

H.verticillata (L.f.) (Hydrocharitaceae) is a submerged aquatic plant native to Asia and Australia and is known to be an invasive species in some parts of the world and maybe a new exotic species in Iraq as it was mentioned in [12]; It is possible that it was introduced during the period of great ecological change that occurred with the drainage and later reflooding of the marshlands of southern Iraq in the 1990s and after 2003. However, it has expanded its distribution as an invasive species in North America, South America, New Zealand, Africa, and Europe [13]. *Hydrilla verticillata* grows in various types of water bodies, at the altitude from 0 to 1600 m above sea level; it prefers lentic, well-warmed, stable level waters, but it can also inhabit lotic water [14].

Ceratophyllum demersum known as coontail is a macrophyte with a worldwide distribution. A completely submerged aquatic plant, it is commonly seen in quiet waters of lakes, ponds, marshes and streams. *C. demersum* is unrooted, free-floating occurs just under the surface of the water, is perennial, and grows quickly. It is capable of forming dense monospecies beds and excluding other plant species [15]. It can cause problems for recreational activities on waterways and can cause blockages at hydroelectric power stations. It can spread rapidly and grow in a large range of aquatic habitats. In Iraq, *C.demersum* is commonly available and grows in rivers, lakes, marshlands, and manmade ponds[16]. It flowers at the beginning of spring. [17]. However, over the last decade, there has been a surge of interest in the response of aquatic plants to the eutrophication in rivers. In this study, we wanted to know the extent to which two aquatic plants respond to eutrophication phenomenon is one of the most widespread environmental problems of inland waters and is their unnatural enrichment with two plant nutrients, phosphorus, and nitrogen [18]. Eutrophic lakes exhibit many undesirable traits, including excessive growth of algae and other aquatic plants. In response to over enrichment with nutrients, the phytoplankton community may shift to bloom-forming nuisance algae, which are harmful to other organisms. as well as the extent to which they compete for nutrients.

On the other hand, a few studies about *Hydrilla* role in aquatic system have shown that this plant could cause problems. Therefore, this study was conducted with the objective of evaluating inter-specific competition between *C.demersum* and *H.verticillata*.

The aim of the current study to know to compete for extent and impact the nutrients (P,N) on some biochemical and physiological criteria of two species of aquatic plants *Hydrilla verticillata* and *Ceratophyllum demersum*.

2. Material and Methods

2.1. Collection of samples and Experiment site

Plant samples of *H. verticillata* and *C. demersum* were collected from populations maintained at the Euphrates River (Al-Zarqa region in Kufa city) located between Longitude °32 99' 861''N and Latitude °44 29' 00''E.(Figure-1). The experiment was carried out in the culture room that included constant temperature and light during the period from May to July (2018) at the Faculty of Science, University of Kufa(Najaf, Iraq, Map1).



Map 1-Map of collect of sample from Euphrates River

2.2. Experimental Design

Both species were cut of terminal shoots into 10 cm length fragments. The existing branches (roots and flower buds) were eliminated. They were brought to the laboratory in nylon bags and well washed with river water. Then, they were washed several times with tap water and distilled water to clean from the dirt and materials attached and remove of the remnants of adherent algae and small river animals. Then, planted for 10 days to adapt the plant to live in the tap water [19]. They were planted in plastic containers all were equal dimensions (40cm length x 25cm width x 25cm height) filled with 15 liters of water per container and water level was in each container maintained at the same level throughout the experiment. Our experiment was designed in 63 containers (plastic tank). divided into five treatments, everyone has four combinations, each combination has 12 containers. In each combinations were only planted without nutrients (control), Remain 3 containers were water control (only water) Table-1.

Our experimental were arranged in a Randomized Design Completely and plant growth parameters (Chlorophyll content, protein content, catalase, superoxide dismutase, and biomass) were recorded after (15, 30, and 45) day of planting after acclimatized of the plant for 14 days in tap water; after acclimation, plant exposed to chosen concentrations of the nutrients; nitrogen (2, 6 and 10) ppm and

phosphorus (0.1, 0.5 and 1) ppm, each 15 days with constant light irrationally (400 Lux.); photoperiod 12/12 light/dark (h./h.) and temperature 30C. Data were analyzed using SPSS statistical software (version 16).

Table 1-Ratio and number of	f terminal shoot	ts of each species	s used per container	in all treatments
	i terminar snoo	to of each species	s used per container	in an acadiments

		Ratio and Number of Plants / Container				
Treatm ent	Combination	Low con. mg/l	Medium con. mg/l	High con. mg/l	Control (0 conc.)	
T1	H.verticillata : C.demersm	10:30	10:30	10:30	10:30	
T3	H.verticillata : C.demersm	20:20	20:20	20:20	20:20	
T2	H.verticillata : C.demersm	30:10	30:10	30:10	30:10	
T4	H.verticillata only	40:00	40:00	40:00	40:00	
T5	C.demersm only	00:40	00:40	00:40	00:40	

- Low concentration = (P 0.1 ppm, NO₃ 2 ppm)
- Moderate concentration = (P 0.5 ppm, $NO_3 6 \text{ ppm}$)
- High concentration = (P 1 ppm, NO₃ 10 ppm)

Forty terminal shoots were planted per container and the method of planting (arrangement of plants in the container). The containers were laid out in a Completely Randomized Design (CRD) representing three replicates per treatment. Hence altogether 2400 terminal shoots 1200 each from *Hydrilla verticillata* and *Ceratophyllum demersum* were used in the experiment.

2.3. Data Analysis

2.3.1. Chlorophyll

Plant growth parameters measured total chlorophyll, chlorophyll a and chlorophyll b according to [20] by spectroscopy type (Spectrophotometer SP-300) at waves (645 and 663) nm.

2.3.2 Proteins

Used the Biuret method to estimate the protein in plant tissues, were measured Absorbance by Spectrophotometer (SP-300) at a 555 nm wavelength. Bovine protein solution was used in the preparation of the standard curve and the protein content was expressed in mg / g plant tissue [21].

2.3.3 CAT enzyme

According to the method [22] was used to estimate the effectiveness of catalase enzyme, then read the absorbance with a wavelength of 240 nm.

2.3.4 SOD enzyme

The used method that according to [23] estimate the effectiveness of the SOD enzyme, and then the optical absorption was read at wavelength 420 nm.

2.3.5 Statically Analysis

The experimental plots were arranged in a Random Design Completely; data were analyzed by using SPSS statistical software (version 16)

3. Results and Discussion

3.1 Total chlorophyll

Our results showed a clear variation in the ratio of total chlorophyll of plants under study, the total chlorophyll was recorded with a maximum value 17.2 μ g/g in (T2) in *H. verticillata* plant at high concertation and the minimum value was 2.45 μ g/g in (T5) in control during 15th days of experimental (Figure-1).

During 30th days of experimental, where record a maximum value in low concertation was 17.87 $\mu g/g$ in T2 of *H. verticillata* and the minimum value was 4.71 $\mu g/g$ recorded in T2 of *C. demersum* at moderate concertation (Figure-2); and during 45th days period of experimental the maximum value recorded was 17.45 $\mu g/g$ in T4 at low concertation and the minimum value was 3.62 $\mu g/g$ in T1 of *H. verticillata* at control (Figure-3).

Statically analysis under probability (p < 0.05) showed there are significant differences between all interactions.

Showed *H. verticillata* higher level of chlorophyll content than *C. demersum* in all combinations at 15 days in low concertation of nitrogen and phosphor. Stress decreases the ability of photosynthetic

systems to utilize incident photons, thus leading to photoinhibition, and reduced quantum yields of photochemistry and chlorophyll fluorescence. Photoinhibition causes inhibition of PSII, while also increasing thermal de-excitation of excited Chlorophyll [24]. The decrease in plant growth may be due to the accumulation of high concentration of nutrients as well as the reduction of pigment content, damage to root cells and deformation of the ultrastructure of chloroplasts and cell membrane [3]. On the other hand, the results of the study showed the highest rate of building chlorophyll pigment in T2 of *H. verticillata*, and the lowest rate of building chlorophyll pigment in the same model but in the other plant, and this may show us, that there is a clear dominance of the *H. verticillata* at the expense of *C. demersum* in this standard.

3.2 Chlorophyll a and b

The average of chlorophyll a of plants in the current study was recorded with a maximum value 4.1 $\mu g/g$ and the minimum value recorded 0.57 $\mu g/g$ during 15th days (Figure-4), and the higher concentration recorded 4.54 $\mu g/g$ and the minimum value was 0.79 $\mu g/g$ during 30th days of experimental (Figure-5), but recorded higher value during 45th days 5.25 $\mu g/g$ and minimum value 0.79 $\mu g/g$ recorded (Figure-6).

While chlorophyll b was recorded high values $14.02 \ \mu g/g$ and minimum value $1.88 \ \mu g/g$ during 15^{th} days of experimental (Figure-7); and the high value recorded was $13.72 \ \mu g/g$ and the minimum value was $3.22 \ \mu g/g$ during 30^{th} days of experimental Figure-8, while recorded higher value during 45^{th} days $13.63 \ \mu g/g$ and minimum value was $2.83 \ \mu g/g$ Figure-9.

Chlorophyll is the major photosynthetic and aquatic plants pigment in a lot of phytoplankton and a trophy index in aquatic ecosystems [25]. Our results of chlorophyll showed a high significant increase in *H. verticillata* compare *C. demersum*, as recorded high value in competing model 10N and 30N. This is due to portability growth of *H. verticillata* in competing with *C. demersum* so showed in high concentration is significant value increase in *H. verticillata* but in *C. demersum* no significant between concentrations. Changes in chlorophyll content can occur as a result of nutrient deficiencies during plant growth. Thereby Chlorophyll contents can be used to manage nutrient optimization programs that both improve crop yield and help to protect the environment [26]. The previous studies confirmation chlorophyll *a* (Chl-*a*) could be predicted as a positive log-linear function of total P [27]. Perhaps the most likely interpretation of our study is that the results of this measurement match the results of the previous measurement (total chlorophyll), The highest construction rates for chlorophyll A were recorded in the *H. verticillata* compared to the lowest construction rates in the *C. demersum*, Where presence a clear dominance of the *H. verticillata* at the expense of *C. demersum* in this standard also.

Either, content of chlorophyll b may increase under pressure, possibly due to increased plant tolerance to stress, and that the plant can adapt to difficult conditions by controlling chlorophyll and a and b [28]. On the other hand, it was observed that the values of chlorophyll b were not changed in the early days of the experiment; however, there was a significant increase in the values of chl.b after reaching the last days under high concentrations, this may lead us to the fact that nutrients play a role in stimulating the buildup of chlorophyll b but slowly, So the effect of phosphorus and nitrogen may need time to show its effectiveness in the plant's body. Generally, the results of chlorophyll b were identical to our previous two measured results (total chlorophyll and chlorophyll A), there has been an evolution in the construction of chlorophyll b at the *H. verticillata*, and in contrast, a reduction in the rate of chlorophyll b construction at *C. demersum*. This congruence in the results between our parameters may raise the validity of the interpretation of the superiority of the *H. verticillata* to a higher degree of emphasis.



Figure 1-Mean total Chl-a values of *C. demersum* and *H. verticillata* after expose to different concentrations of nutrients During 15th days of experimental.

Where: * T1= 10H:30C, T2=20H:20C, T3=30H:10C, T4=40H and T5=40C. H=High concentration mean (N=10 ppm, P=1 ppm) M= Moderate concentration mean (N=6 ppm, P=0.5 ppm) and L= Low concentration mean (N=2 ppm, P=0.1 ppm).



Figure 2-Mean total chlorophyll values of *C. demersum* and *H. verticillata* after expose to different concentrations of nutrients During 30^{th} days of experimental.



Figure 3-Mean total Chl-a values of *C. demersum* and *H. verticillata* after expose to different concentrations of nutrients During 45^{th} days of experimental.



Figure 4-Mean Chl-a values of *C. demersum* and *H. verticillata* after expose to different concentrations of nutrients During 15 days of experimental.



Figure 5- Mean Chl-a values of *C. demersum* and *H. verticillata* after expose to different concentrations of nutrients During 30th days of experimental.



Figure 6-Mean Chl-a values of *C. demersum* and *H. verticillata* after expose to different concentrations of nutrients During 45th days of experimental.



Figure 7-Means of Chl-b of *C. demersum* and *H. verticillata* after expose to different concentrations of nutrients during 15 days of experimental.



Figure 8-Means of Chl-b of *C. demersum* and *H. verticillata* after expose to different concentrations of nutrients during 30th days of experimental.



Figure 9-Means of Chl-b of *C. demersum* and *H. verticillata* after expose to different concentrations of nutrients during 45th days of experimental.

3-3 Total protein

Clearly Figure-10, the mean total protein of study plants during 15th days of experimental showed a maximum value was 14.75 mg/g in (T2) of C. demersum plant at moderate concentration and the minimum value was 6.4 mg/g in (T3) of C. demersum at high concentration; and the higher concentration recorded in (T5) in moderate concentration was 16.17 mg/g and the minimum value was 4.17 µg/g in moderate concentration of *H. verticillata* in T3 during 30th days of experimental (Figure-11); but recorded higher value during 45th days was 12. 4 mg/g in *H. verticillata* in (T2) at control and minimum value was 3.58 mg/g recorded (T4) at high concentration (Figure-12). The results of the statistical analysis showed significant differences in the probability level (p < 0.05) between all interactions. The decrease in protein content of plant during experimental period is maybe due to plant stress for the formation of ROS (Reactive Oxygen species), which is an oxygen- containing chemical reaction molecule such as hydrogen peroxide, Superoxide anion (O_2) , and hydroxyl radical (OH), leading to a oxidative stress that produces these compounds as transverse products during metabolism that effect in plant cells and lead to their death as well as the breakdown of protein, fat, and DNA [29]. The increased nitrogen rates resulted in a significant decrease in protein content as opposed to the increased potassium rates. An opposite relationship was found in earlier studies [30]. On the other hand, in other study on Eruca vesicaria grew during the autumn season accumulated protein in a similar amount, regardless of increasing nitrogen rate [31]. Although decrease the protein content of both plants is generally under various nutrient concentrations. But, the current study showed the highest content of protein when C. demersum grows alone were recorded the highest value in (T5) with 16.17 mg/g while the less content appeared when the *H. verticillata* grows alone where recorded the lowest value in (T4) with 3.58 mg/g, While in the interference treatments, a significant reduction in protein content was observed in C. demersum offset by a significant increase in the protein content of the *H. verticillata*. This may lead to the belief that the growth of the *H. verticillata* alongside the *C.* demersum inhibits protein synthesis in the latter.



Figure 10-Means of total protein of *C. demersum and H. verticillata* after expose to different concentrations of nutrients during 15th days of experimental



Figure 11-Means of total protein of *C. demersum and H. verticillata* after expose to different concentrations of nutrients during 30th days of experimental



Figure 12- Means of total protein of *C. demersum and H. verticillata* after expose to different concentrations of nutrients during 45th days of experimental

3-4 CAT enzyme

Our results catalase enzyme values effectiveness recorded a maximum value in C. demersum was 53.3 units /mg in (T3) at control treatment, and the minimum value recorded in *H. verticillata* was 10 units/mg in (T2) in low concertation during 15 days of experimental (Figure-13), and the higher value recorded in *H. verticillata* in (T1) was 53.3 units /mg in high concertation and minimum value was 11.6 units /mg in the (T5) at control treatment during 30th days of experimental Figure-14, during 45th days recorded higher value of catalase enzyme effectiveness in (T1) of H. verticillata at control treatment was 50 units /mg and minimum value recorded in (T1) of C. demersum was 10 units /mg in high concertation Figure-15. Results of the statistical analysis showed significant differences in the probability level (p<0.05) between all interactions excepted plant and experimental period no significant. Enzyme activity increase during the 30 days and it decreased in the 15 days of the experiment may be due to the plant's susceptibility to possibly stress conditions for the period or maybe to the role of high concentration in simulating the bioprocessing of antioxidant enzymes [32]. In this study, the efficacy of the CAT enzyme was a good indicator of the competition between the two plants, the early days saw the dominance of the C. demersum and the rise in the values of the CAT against the weak efficiency of the *H. verticillata*; However, after 30 days, the CAT in the *C. demersum* appeared to be down to less effective, while the *H. verticillata* regained its activity and scored a clear dominance in the last days of the experiment. Enzyme activity decreased during the 15th day and it increases in the 30th day of the experiment, may be due to the plant's susceptibility to possibly stress conditions for the period or maybe to the role of high concentration in simulating the bioprocessing of antioxidant enzymes [33].



Figure 13-Means of catalase enzyme effectiveness of *C. demersum and H. verticillata* after expose to different concentrations of nutrients during 15 days of experimental.



Figure 14-Means of catalase enzyme effectiveness of *C. demersum and H. verticillata* after expose to different concentrations of nutrients during 30th days of experimental



Figure 15-Means of catalase enzyme effectiveness of *C. demersum and H. verticillata* after expose to different concentrations of nutrients during 45th days of experimental

3-5 Super Oxide dismutase (SOD) enzyme

SOD enzyme values recorded high value in C. demersum were 0.24units/mg in (T2) at control and the lowest value recorded in the H. verticillata in (T4) was 0.012 unit/mg at moderate and high concertation during 15th days of experimental (Figure-16) while the higher value recorded in low concertation was 0.053 unit/mg in T1 of H. verticillata and minimum value recorded in high concertation was 0.011 unit/mg in (T5) during 30th days of experimental (Figure-17), during 45th days of experimental recorded higher value was 0.077 unit/mg in (T3) of C. demersum at moderate concertation while minimum value recorded was 0.010 unit/mg in low concertation (Figure-18). The results of the statistical analysis showed significant differences in the probability level (p<0.05) between all interactions. The high efficiency of the enzyme in the leaves is similar to the leakage of electrons from the chain of transmission of electrons in photosynthesis to the oxygen molecule [34,35]. The results of the current study showed a gradual decreased in the SOD values and attributed the reasons for the effectiveness of the enzyme may be due to the fact that the antioxidants of the enzyme have differed in their effectiveness [35]. The low efficiency of the enzyme may be due to the sensitivity of the plant to high concentrations that reduce its effectiveness and thus lead to the collection of ROS in plant tissues and increase the rate of DNA destruction [36]. In the competitive aspect between the two plants, there was a common dominance between the two plants throughout the experiment, except for the last few days, Appeared the superiority of the C. demersum in (T3). This may explain the possibility of C. demersum to stimulate the increased production of the enzyme SOD when it's under stress.



Figure 16-Means of SOD enzyme of *C. demersum and H. verticillata* during 15th days of experimental



Figure 17-Means of SOD enzyme of *C. demersum and H. verticillata* during 30th days of experimental



Figure 18-Means of SOD enzyme of *C. demersum and H. verticillata* during 30th days of experimental

3-6 Biomass

Values of biomass recorded a high value in *C. demersum.* were 34.4g at (40N) in low concentration and the lowest value recorded in the *H. verticillata* at 10N in moderate concentration was 2.1g. Results of the statistical analysis showed significant differences in the probability level (p<0.05) between all interactions excepted plant and number no significant. (Figure-19). High biomass appeared in *C. demersum* compare to *H. verticillata*, due to a probability of *C. demersum* to grow faster than *H. verticillata* plant in low and moderate concentration only. In contrast to [37]. increase in biomass or area submerged aquatic plants, came from invasive species (*H. verticillata*), which may not provide the same ecological benefits as native freshwater species.



Figure 19-Means of Biomass of *C. demersum and H. verticillata* after expose to different concentrations of nutrients during experiment period

4. Conclusion

Both *Hydrilla verticillata* and *Ceratophyllum demersum* could live in a similar ecosystem conditions, However; if coexist both plants, will compete for the similar nutrients (N,P). Based on the present study. In a situation where *Hydrilla* and *Ceratophyllum* coexist, *H. verticillata* will have a competition advantage over *C. demersum* in our parameters, are necessary increased in *Hydrilla*,

whereas it was significantly reduced in *Ceratophyllum*. Excepted, SOD enzyme (under limited conditions) and Biomass parameters. It recorded the opposite results where *C. demersum* will have a competitive advantage over *H. verticillata*. But under high concentrations of nutrients showed the biomass superiority of the *H.verticillata* on the *C.demersum*; This may explain the possibility that the *H.verticillata* adapts to living under eutrophication conditions greater than *C.demersum*. Thus, leads us to that, *H. verticillata* could superiority on *Ceratophyllum demersum* in most competition standards, This probably explains the reason for the spread *Hydrilla verticillata* in most of the water bodies in Iraq.

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