Egypt. J. Aquat. Biol. & Fish., Vol. 4, No.1 :125-138(2000) *ISSN 1110-6131*

IMPACT OF UREA FERTILIZER ON GORWTH AND BIOCHEMICAL COMPOSITION OF SOME AQUATIC PLANTS

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(Received November 13, 1999)

Key words: Aquatic plants, crude protein, fat content, yield, urea.

ABSTRACT

This study has been conducted to evaluate the effect of varying concentrations of urea fertilizer on the growth, protein, fat and water content of five aquatic plants, in aquaculture system. These plants were cultured in glass basins outside the laboratory in tap water and they received doubling doses of urea fertilizer (1, 2, 4, 6, 8 and 16g) per 30 L medium at three days intervals. The media were changed before receiving the following inoculum and the excess yields were harvested. *Potamogeton crispus*, *P. pectinatus* and *Ceratophyllum demersum* could withstand urea concentrations up to 533 mg/L, while *Polygonum amphibium* could survive at 133 mg/L, but *Azolla filiculoides* died at the same concentration. Crude protein increased, generally during the experiment, in all test plants except in *P. crispus* that showed wide fluctuations. Fat content decreased in all plants by the end of the experiments, but water content fluctuated slightly between increases and decreases during the experiment.

INTRODUCTION

All waters used for irrigation contain various amounts of soluble or dissolved salts. Some of the individual constituents of the dissolved salts may be beneficial to plant growth and other are harmful. The variability and complexity of many effluents make it difficult to assess

their impact on aquatic organisms in the receiving water. However, little is known about the responses of aquatic plants to various combinations or varying concentrations of chemicals.

Thus, the effects of many chemicals on aquatic plants under laboratory test conditions, should be tested. This, although aquatic plants in the receiving water may be subjected to many more stresses than those tested under laboratory conditions. Urea contains approximately 45 percent nitrogen and is the most commonly available and lowest cost nitrogenous fertilizer. Urea is the most efficient form of nitrogen supply to terrestrial crops, but its volatility in water and its elevating effect on pH makes it problematic for hydroponic applications. Skillicorn *et al.* (1991) suggested that 4.5 kg urea/ha/day were enough to sustain a yield of 1,000 kg/ha/day of fresh duckweed.

Many authors have attempted to utilize or determine the potential usefulness of aquatic plants that has cost nothing to grow, and which is relatively easy to harvest because floating or submerged plants are usually fragile. On the other hand, technology required for production, harvesting and processing has not yet been well developed.

Skillicorn *et al.* (1991) suggested that aquatic plants would be important as a source of fish and poultry feed and simultaneously as a wastewater treatment process in selected areas of the Middle East, particularly in Egypt. they claimed also that, aquaculture systems are many times more productive than terrestrial agriculture and have the potential to increase protein production at rates similar to increases of terrestrial carbohydrate crops. In addition, the solid fraction of most aquatic plants has about the same quantity and quality of protein as soybean meal.

Limaza *et al.* (1984), tested some aquatic plants and decided that they contained more protein than corn. In addition, Dewanji (1993), indicated that leaf protein extracted from unwanted aquatic plants could

be used for food / feed purposes. Even fibrous biproduct, left after the extraction of aquatic leaf proteins, could be utilized as additional feed for ruminants.

Van Hove and Leieune (1996) outlined the useful characteristics of *Azolla*, thus it can be utilized for rice production, as a green manure to supply feed for animals and fish, and a nitrogen fertilizer to improve soil structure.

However, growth of aquatic plants may well serve the dual function of extracting nutrients from wastewater effluents and producing animal feeds. The economic value of the harvested plants may totally or partially compensate for the cost of nutrient removal (Culley and Epps, 1973).

Several criteria considered in evaluating aquatic plants for the above purposes included selection of plants that; (a) could be easily harvested, (b) were low in water content, (c) had high protein content, (d) had low fiber content, (e) had extended growing and harvesting periods, (f) were non-toxic to humans and domestic stocks, (g) were capable to being processed. Some species of submerged, emerged and floating plants seem to fulfill some of the criteria. From them, *Potamogeton pectinatus, P. cripus, Ceratophyllum demersum, Polygonum amphibium* and *Azolla filiculoides.* The purpose of this research is to investigate the biomass production, of the 5 aquatic plants (submerged, emergent and floating) grown on water contaminated with urea fertilizer, and estimate the commercial possibilities of the test plants.

MATERIALS AND METHODS

Plant sample :

All plants were collected during the period between October (1998) and April (1999). Fresh plants were taken from Abis waterways,

near Alexandria city, but away from waters receiving direct run-off from croplands. Occasionally pure stands of the test species were collected for the experiments. All samples were thoroughly washed with tap water. Fresh and young plants were used for the experiments.

The widespread species; the submerged *Potamogeton pectinatus* L., *P. crispus* L., *Ceratophyllum demersum* L., the free floating *Azolla filiculoides* and the emergent *Polygonum amphibium* were planted in glass basins (30L capacity) outdoors, on chlorine free tap water, (1g fresh plant/ L medium). Tap water left in big jars 3 days to get rid of chlorine.

Experiment :

To test the effect of nitrogen fertilizer on growth and internal composition (nitrogen and fat) of the plants. Different combinations of nitrogen in the form of urea fertilizer were added. All culture media were inoculated with urea concentrations of 1, 2, 4, 8, 16 and 32g urea fertilizer, at a period interval of 3 days. One gram urea contained 0.46g N. The whole media were changed every three days before receiving the following urea enrichment. Three replicates were used per species per treatment. The initial plant inoculum was maintained (1g/ L medium) by harvesting the excess yield, which is considered as a growth indicator.

Analysis

Fresh plant samples were weighed and then dried at 105^{o}C for 24 hours. After complete dryness their weights were determined and the differences were calculated for water content. The dry samples were used for determination of total nitrogen and fat contents. Total nitrogen was determined after grinding the plant material and digestion with $K_2Cr_2O_7$/ H_2SO_4 (Golterman *et al.*, 1978). Fat content (ether extract) was determined using soxlett apparatus and petroleum ether was used as ogranic solvent (A.O.A.C.,1990).

RESULTS AND DISCUSSION

The dry yield of *Potamogeton crispus* fluctuated between 0.113 and 0.310g / g initial and between 0.094 and 0.139g/g initial for *P. pectinatus*. *Ceratophyllum demersum* gave yield between 0.082 and 0.136g / g initial dry weight. It appeared that *P. crispus* outperformed the other two plants attaining highest biomass yield within 15 days incubation, but they all could withstand high N-urea concentration in the substrate up to 533 mg/ l medium. Higher N-urea inoculum lead to disintigration of the three plants (*P. pectinatus*, *P. crispus* and *C. demersum*) as shown in table 1.

Rogers and Breen (1980) mentioned that, nitrogen appeared in least supply during growth of *P. crispus*, and may have been limiting. In the current experiments enrichment of N-urea fertilizer at concentration level of 133 mg/l proved to enhance growth of *P. crispus* with a biomass yield 2.5 times the initial plant inoculum. Higher N-urea concentrations yield lower biomass. *P. pectinatus* and *C. demersum* needed lower nitrogen addition (33 mg N-urea/ L) to increase their biomass production by about 1.2 times their initial inoculum. Higher N-urea addition lead to slight but consequent decreases in their yield (Fig. 1).

Wijck *et al.* (1994) pointed out that 100mg N-urea improved biomass production of *P. pectinatus*, but 400 mg N-urea yielded higher biomass. The previous authors tested the plant for N-urea enrichment but in sediments used for plant growth. Thus, nitrogen was available for both roots and shoots. However, *P. pectinatus* grows mostly from tubers and can therefore establish itself rapidly, even in systems with a low nutrient. Additionally, the denitrifying capacity of epiphyton, on shoots of *P. pectinatus*, adapted to high nutrient loadings was about 100 times higher than that of epiphyton adapted to lower nutrient levels (Erikson and

Weisner (1996). This could explain also, the adaptability of *P. pectinatus* to the N-urea concentrations which reached 533 mg/ l medium.

Goulder and Boatman (1971) showed that growth of *C. demersum* was affected by the nitrogen supply and good growth was obtained in experiments by adding nitrogen (~ 4mgN / L). *C. demersum* gave best yield at 33mg N-urea/ L, but the yield was less pronounced by additional N-urea.

Polygonum amphibium is said to indicate low levels or the absence of pollution (Whitton, 1975), but it seemed that it could tolerate N-urea levels up to 67 mg/ L. Higher concentration lead to a detach of the green leaves and wilting of the stems.

Concentration of 33 mg N-urea enhanced *Azolla filiculoides* growth, but 67mg/L lead to decreased growth accompanied by detach of its roots and 133 mg/ l was lethal to the plant. These results were in accordance with Manna and Singh (1990) who indicated that increasing levels of urea nitrogen decreased the fresh biomass of *Azolla* spp.

Crude protein was variable in the 2 *Potamogeton* species and *Ceratophyllum* but consistantly higher than their initial in *P. pectinatus* and *C. demerum* ranging from 23.8 to 30.3% and from 25.3 to 39.8% respectively (Fig. 2). It decreased in *P. crispus* compared to its initial, after treatment, to a range from 23.3 to 36.2%. Van Vierssen (1982) found a maximum nitrogen concentration of 4.1% (25.23% protein) in *P. pectinatus*, but this was still lower than obtained in the present investigation. Also, Balley (1965) gave protein content 18.3% in *Ceratophyllum* sp. considering its commerical possibilities, as new additives which could improve the efficiency of feed demands.

Crude protein reached 19.41% in dry tissues of *P. amphibium* from 11.61%. This values was less than given by Byers (1961) for *Polygonum* sp. used for protein extraction (24.13%). *Azolla* protein

increased slightly (from 22 to 24.94%) after 6 days incubation period. Similar result was given by Kitoh and Shiomi (1991), who mentioned that urea addition in the medium resulted in an increase in nitrogen content in *Azolla.*

It is important to point out that it cannot be assumed that all nitrogen goes into protein, because there is non-protein nitrogen (amino acids) which may compose about 30% of the dry weight of aquatic plants (Culley and Epps, 1973).

The concentration of protein in both *Potamogeton* spp., *Ceratophyllum demersum* and *Azolla filiculoides* dry tissues compared favorably with that in many animal feeds (Alfalfa meal, yellow corn, soybeans and wheat bran contained 20.0, 8.8, 37.0 and 14.5% crude protein respectively).

Fat content dropped in all test plants by the end of the experiments. Fat content in the initially used test plants represented respetcively; 0.71, 0.55, 0.37, 5.5 and 0.22% in *P. crispus, P. pectinatus, C. demersum, A. filiculoides* and *P. amphibium.* It dropped to 0.61, 0.42, 0.29, 3.9, and 0.23% respectively. Fat values in the tested plants were generally lower than those of animal feeds, with the exception of *Azolla filiculoides*. Compared with fat composition used in animal feeds (alfalfa meal, corn grass, soybean meal and wheat bran which contained 3.5, 3.8, 3.5 and 3.0% respectively), *Azolla filiculoides* showed favorable levels, even after exposure to pollution stress by urea fertilizer.

Water content of the different test plants did not exhibit pronounced variations. It varied from 86.4-89.5%, 88.9-90.6%, 90.4-29.1% and from 83-86.6% in *P.crispus, P. pectinatus, C. demersum* and *P. amphibium* respectively, while very slight decrease in water content was observed in *A. filiculoides* (from 85.7 to 85.0%). Joy (1969) reported

values of 86 to 89% obtained in plants having poor growth rates. He suggested that the lower water content was caused by more starch in the plants, which may be an advantage because starch is a major source of energy in feedstuffs.

In conclusion, N-urea could be a limiting factor for growth of some plants (*Azolla* and *Polygonum*) in waterways which may receive the agricultural drainage contaminated with urea fertilizer. On the other hand, these drainage water could be advantageous for other macrophytes growth, with extended growing and harvesting periods.

The present study also concluded that some of the test plants (*P. crispus, P. pectinatus* and *C. demersum*) had promising possibility for protein extraction and possible utility in manufacturing of food for fish and animals.

Aquatic plants farming can be a continuous process, requiring intensive management for optimum production. Harvested plant biomass can be used daily in its fresh form as fish feed or dried for use in other animal feeds.

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Table 1. Growth characteristics, dry matter yield, % water content, %crude protein and % Fat (ether extract) during the experiment in the different test plants, cultured outdoors in media enriched with different combinations of N-urea.

Plant species	Duration of incubation days	N-urea concentration mg/l	Dry weight (g/ g initial)	% water content	% crude protein	% fat (ether extract)
Potamgeton crispus	Start	—	0.125	87.5	39.13	0.71
	3	33	0.146	87.5	27.44	N.E
	6	67	0.185	86.5	25.00	N.E
	9	133	0.310	88.6	36.19	N.E
	12	266	0.145	89.5	23.25	N.E
	15	533	0.113	86.4	25.25	0.61
P. pectinatues	Start	—	0.113	88.7	16.75	0.55
	3	33	0.139	88.9	25.00	N.E
	6	67	0.115	90.1	30.32	N.E
	9	133	0.129	88.9	28.37	N.E
	12	266	N.E.	N.E.	N.E.	N.E
	15	533	0.094	90.6	23.83	0.42
Ceratophyllum demersum	Start	—	0.109	89.1	23.75	0.37
	3	33	0.136	90.4	25.28	N.E
	6	67	0.135	91.4	39.79	N.E
	9	133	0.117	91.6	30.90	N.E
	12	266	0.093	92.1	27.70	N.E
	15	533	0.082	91.8	33.24	0.29
Polygonum amphibium	Start	—	0.170	83.0	11.61	0.22
	3	33	0.163	N.E.	N.E.	N.E
	6	67	0.140	N.E.	N.E.	N.E.
	9	133	0.136	86.6	19.41	0.23
Azolla filiculoides	Start	—	0.137	85.7	22.00	5.50
	3	33	0.144	85.7	27.51	N.E
	6	67	0.141	85.0	27.51	N.E.
	9	133	Distintigrate	—	—	3.9

N.E. not estimated because the dry yield was very small.

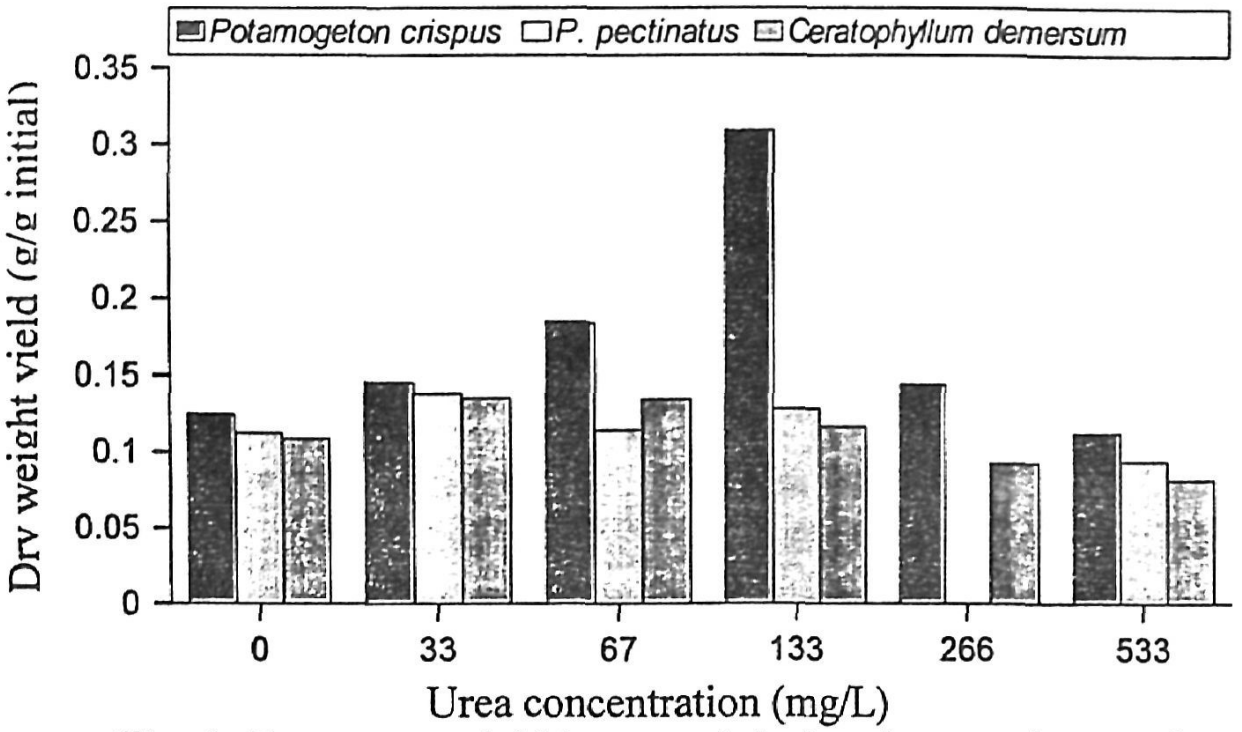

Fig. 1: Dry matter yield harvested during the experiment after enrichment with N-urea fertilizer.

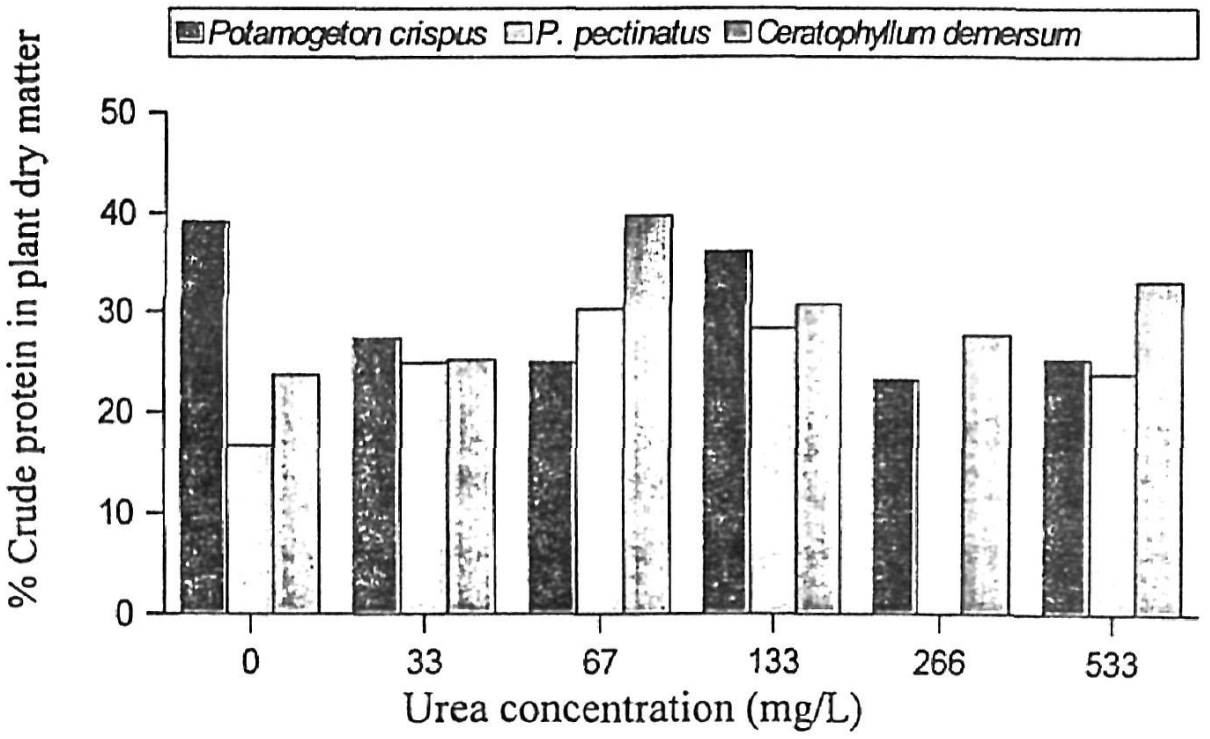

Fig. 2: Fluctuations in % crude protein in the test plants as affected by variations in N-urea concentrations.