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RESPONSE OF BARLEY FORAGE PRODUCTION AND CHEMICAL ANALYSIS TO ADDING *AZOLLA* UNDER HYDROPOIC SYSTEM

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ABSTRACT

Barley (Hordeum vulgare L.) matures faster and more evenly under a hydroponic system than a conventional soil based system. Supplemented Azolla (Azolla Pinnata) to hydroponic system as an aquatic free floating fern provides protein with all essential amino acid which is required for animal nutrition. So, this investigation aimed to evaluate green fodder production and inhibition of root rot fungi under hydroponic conditions for barley. The experiment has been conducted under temperature and artificial illumination at growth room of Soilless Culture Laboratory, Sadat city University, Egypt. The results showed that green fodder can be produced in 8 days using hydroponic technique. Trays treated with Azolla gave more green fresh yield (11.8 kg/tray) than untreated ones (7.2 kg/tray). Adding Azolla increased of protein, N, P and K content percentages by 28.7, 0.98, 0.82 and 0.70 %, respectively, as compared to no addition of Azolla. There is no disease incidences were achieved with treated by Azolla as compared to 26.0 % with those untreated by Azolla. It could be concluded from this study that adding Azolla to hydroponic barley forage production improved green forage yield and its chemical analysis.

Keywords: Hydroponic barley, Azolla, root rot fungi.

الملخص العربي

الهدف من إجراء هذه الدراسة هو تقييم استجابة انتاج العلف الأخضر من الشعير والتركيب الكيميائى بإضافة الأزولا لبيئة النمو تحت نظم الزراعة المائية ودور ذلك فى تثبيط فطريات العفن الجذرية تحت ظروف الزراعة المائية. حيث تم إجراء التجربة تحت ظروف ضبط الحرارة (٢٥ ± ١ درجة مئوية) والإضاءة الأصطناعية في غرفة أستنبات مختبر الزراعة بدون تربة (المائية)، معهد الدراسات والبحوث البيئة, جامعة مدينة السادات ، مصر. وأظهرت النتائج أن العلف الأخضر يمكن إنتاجه خلال ٨ أيام من بداية الزراعة إلى الحصاد باستخدام تقنية الزراعة المائية. تم تسجيل أعلى قيم لمحصول العلف الأخضر الطازج في معاملة أستنبات الشعير باستخدام الآزولا حيث بلغ ١١,٨كجم / صينية، مقارنة بمحصول العلف الأخضر للشعير المستنبت والذي لا تحتوي على الأزولا حيث بلغ ٧,٢ كجم / صينية, وكذلك أظهرت النتائج أن النسب المئوية لمحتوى محصول العلف الأخضر للشعير المعالج بـ الأزولا من الـ N، P، K والبروتين، كانت أعلى بشكل ملحوظ وبنسب بلغت ٠,٩٨ ، ٠,٨٢ ، ٠,٧٠ و ٢٨,٧٪ على التوالي من تلك الغير معالجة بالأزولا.

وكذلك من أهم النتائج المتحصل عليها عدم حدوث أي نسبة إصابة مرضية بعفن الجذور في حالة النباتات المعالجة مع الآزولا, مقارنة مع النباتات الغير معالجة بالآزولا حيث بلغت نسبة الإصابة بعفن الجذور ٢٦%. يمكن أن نستخلص من هذه

الدراسة أن محصول الشعير العلفي يمكن أعتباره الاختيار الأفضل لإنتاج الأعلاف الخضراء المائية باستخدام الآزولا التي بها نسبة عالية من البروتين وخالية من فطريات أعفان الجذور.

- **INTRODUCTION**

As livestock population increases, large gap exists between requirements and availability of feed to maintain animal nutrients requirement. Studies have shown that production of forage crops covers about 20-25% of its livestock feed requirements (**Harb and Awawdeh, 2008**). It is a fact that feeding animals is deficient without including green fodder (e.g. Green forage) in their diets (**Shah *et al.,* 2011**). Green fodder is an essential component of the livestock ration to enhance their productive and reproductive performance. Consequently, for improving livestock products, quality green fodder should be fed more often to animals (**Dung *et al.,* 2010**). However, the major constraints in production of green fodder are decreasing land size for fodder cultivation, scarcity of water, labor requirement, and high cost of fertilization (**MOA, 2014**). As a solution, growing hydroponics fodder may control some livestock feed diets and improve performance (**Rodriguez *et al.,* 2004**).

Fodder production is accelerated by as much as 25% by bringing the nutrients directly to the plants, without developing large root systems to seek out food. Plants mature faster and more evenly under a hydroponic system than a conventional soil based system (**Bakshi *et al.*, 2017**). One kg of un-sprouted seed yields 8-10 kg green forage in 7-8 days (**Sneath and McIntosh, 2003 and Naik *et al.*, 2013**). The hydroponics maize fodder yield on fresh basis is 5-6 times higher than that obtained in a traditional farm production, and is more nutritious (**Naik *et al.*, 2014**).

Hydroponics is an advanced technology in agriculture. These techniques can meet the growing nutrients requirement for livestock feed with suitable prices, in addition to guarantee a constant production of high quantity of green forage throughout the year. Hydroponic fodder production is a technique of growing crops such as barley, in a hygienic environment free of chemicals, and artificial growth promoters (**Jensen and Malter, 1995**). Hydroponically fodder has a short growth period (around 7-10 days) and requires a small piece of land for production (**Mooney, 2005**). It has high feed quality that is rich with proteins, fibers, vitamins, and minerals (**Bhise *et al.,* 1988 and Chung *et al.,* 1989**) with health beneficial effects on animals (**Boue *et al.,* 2003**). As a reason, hydroponic culture is one of the most important agricultural techniques currently in use for green forage production in many countries. This technique can be used for green fodder production of many forage crops in a hygienic environment free of chemicals like insecticides, herbicides, fungicides, and artificial growth promoters (**Jensen and Malter, 1995 and Al-Karaki and Al-Momani, 2011)**. It is a well-known technique for high fodder yield, year round production and least water consumption (**Al-Karaki, 2010**). Unlike field production system that use run-to-waste irrigation practices, the hydroponic fodder system uses recirculation system, thus reducing the waste water. It has been reported that hydroponic fodder production requires only about 2-3% of that water used under field conditions to produce the same amount of fodder (**Al-Karaki and Al-Momani, 2011)**. Fodder produced hydroponically is of a short growth period 7–10 days and does not require high-quality arable land, but only a small piece of land for production to take place (**Cuddeford, 1989 and Mooney, 2005**). It is of a high feed quality, rich with proteins, fiber, vitamins, and minerals (**Lorenz, 1980 and Bhise *et al.,* 1988**).

The aquatic pteridophyte Azolla is an excellent biofertilizer and green manure having global distribution. Ability of *Azolla-Anabaena* system to fix atmospheric nitrogen at faster rates makes it an outstanding agronomic choice for the cultivation of rice under tropical conditions. Nitrogen fixation potential of the *Azolla-Anabaena* system has been estimated to be 1.1 kg N ha^{-1} day^{-1} and one crop of Azolla provided 20-40 kg N ha^{-1} to the rice crop in about 20-25 days (**Watanabe *et al.,* 1977**). The ability of nitrogen fixation is due to the presence of a *heterocystous cyanobacterium Anabaena Azollae* which is confined to the dorsal leaf cavity of the fern (**Moore, 1969**). This cavity is formed by the enfolding of the adaxial epidermis in the dorsal leaf lobe. Azolla has multifaceted uses and has gained considerable importance in the recent times as biofertilizer, green manure and as poultry feed and cattle fodder increase in body weight of the chicks upon feeding with Azolla was observed. It is a rich source of protein and essential amino acids and contains several vitamins such as vitamin-A, vitamin B-12 and beta carotene. Also, it's rich in minerals such as Calcium, Phosphorous, Potassium, Magnesium, Copper and Zinc….etc. The protein composition of Azolla is 25-35% on dry weight basis and is easily digested by poultry (**Parashuramulu *et al.,* 2013**). Azolla has many uses such as animal feed, human food, medicine, production of biogas, hydrogen fuel, water purifier, weed control, reduction of ammonia volatilization and is aptly referred it as green gold mine (**Wagner, 1997**). Azolla plants in huge numbers sequestered significant quantities of atmospheric CO_2 and converted it directly into biomass of Azolla (**Speelman *et al.,* 2009**). Azolla filiculoides was also used in diets for sows (**Leterme *et al.*, 2010**). Also, it can be considered as potential unconventional feed for livestock; contains 82.66 % organic matter, 22.48 % crude protein, 4.5 % ether extract, 14.7 % crude fiber and 40.98 % nitrogen free extract as well as the total Ash content was 17.34 % (**Anitha *et al*., 2016**).

So, the objectives of this study were to evaluate efficiency using Azolla in production of barley green fodder with high protein and free from root rot fungi under hydroponic system.

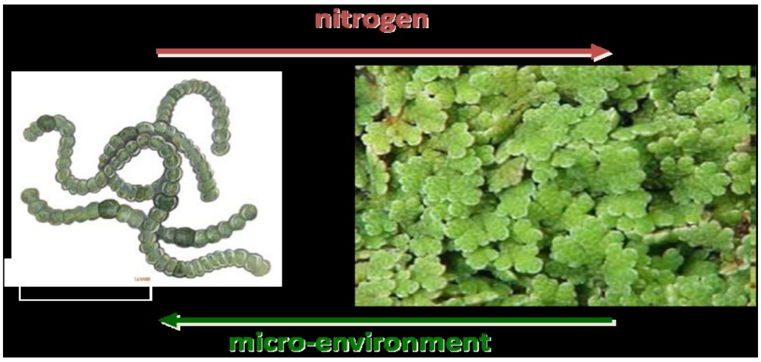

Fig. 1: *Azolla-Anabaena* system to fix atmospheric nitrogen

1- MATERIALS AND METHODS

This study was conducted under room temperature (25±1°C) for all months of the year and artificial illumination at growth room of Soilless Culture Laboratory, Sadat city University, Egypt. A hydroponic system composed from metal frame was designed and assembled from local materials has been used in this study.

2.1. THE HYDROPONIC SYSTEM.

The hydroponic system is composed of two cabinets (units) with metal frame and four shelves each with a length of 200 cm, a width of 72 cm, and a height of 240 cm. Each unit of the system could carry 25 planting trays with capacity to produce approximately 80-100 kg green fodder per growth cycle (8 days), depending on crop variety and growth conditions (**Al-Karaki and Al-Momani, 2011**). The horizontal area occupied by each unit of the system was about 2 m^2 including the pathways between neighboring units. However, the number of units of the hydroponic system can be increased and planting date scheduled for daily production of green fodder to meet the daily demand of animals in the farm. Polystyrene trays with a length of 70 cm, a width of 30 cm and a depth of 5.8 cm were used for growing seeds to produce green fodder. These trays were obtained from the local market. The units of hydroponic system have been arranged in the growth room with artificial illumination. Under room temperature was used to control temperature inside the growth room. The relative humidity in the growth room ranged between 50 and 73%.

2.2. PLANT MATERIAL.

Forage crop was evaluated in this study which was: barley (*Hordeum vulgare* L.), Seeds of this crop was obtained from the local market of Cairo city, Egypt. Seeds of the barley crop were subjected to a germination test to check for their viability before being used; the germination percent was 73%.

Fig. 2: The hydroponic system in which planting trays were stacked after seeding of barley crop.

2.3. TREATMENT OF SEEDS BEFORE PLANTING.

Seeds of barley were cleaned from debris and other foreign materials. Then seeds soaked in tap water overnight (about 12 hours) before planting.

2.4. SEED PLANTING AND IRRIGATION.

First treatment trays, soaked barley seeds germinated upon fresh Azolla which was as lower surface, but the second treatment trays (control) kept without Azolla. All trays were lined with plastic sheets and have holes at the bottom to allow drainage of excess water from irrigation. The seeding rates were about 450 g/ tray. The trays were stacked on the shelves (25 trays/5 shelves) (**Ghazi *et al.*, 2012**). Trays were irrigated manually with tap water twice a day (early in the morning and late in the afternoon) at a fixed rate of 500 ml/tray/ day which was enough to keep the seeds/seedlings moist Figure 3 and 4.

(A) (B)

Fig. 3: (A) *Azolla* spread at low surface, (B) seeds of barley

(A) (B) (C)

Fig. 4: Subsequent growth of barley seedling (A) First day of barley seeds, (B) 2^{nd} day growth (C) 3^{rd} day growth

2.5. FORAGE YIELD:

The experiment was terminated after 8 days from seeding, where the fodder biomass was ready for harvest (Fig 5). At harvest time, the following data were recorded per tray. Total fresh and dry fodder yields, seedling height, leaf colour, percentage of N, P, K and protein content. Also the disease incidence (DI) $\frac{\text{no of wilting plants}}{\text{total number of plant}} \times 100$ were recorded to show percentage of root rots fungi, according following formula (Trapero-Casas and Jimenez Diaz, 1985). DI as the presence or absence of disease (percentage of infected leaves on the plant). A representative fresh plant samples (about 150 grams) from every tray were taken at harvest, oven-dried at 70°C for 48 hours, and weighed to compute the moisture content.

Fig 5: Green fodder of barley crop ready for harvest.

2.6. EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS.

Completely randomized design was used with three replicates. Data were statistically analyzed using T-test in groups using MSTATC program. Means separation were tested by LSD ($P \leq 0.05$).

1. RESULTS and Discussion

Results showed vegetative growth parameters and green forage production of barley after 8 days from planting (Table 1, 2, 3, 4 & Fig. 6)

Barley seedlings treated with *Azolla* were significantly higher (24 cm) than those of without *Azolla* treatment (18 cm). According to leaves color, seedling treated with *Azolla* had dark green color leaves.

Table 1: Response of barley growth parameters to *Azolla* addition under hydroponic system over replicates.

Treatments	Plant height (cm)	Leaf color
Control	18 b	Green
Treated	24 a	Dark green

T-test in groups and LSD at p= 0.05 level of significance.

Fig 6: Green forage yield of barley with low surface layer of Azolla

. The results in Table 2 revealed that the fresh weight of barley fodder/tray in case of plants treated with *Azolla* were higher (11.8 kg/tray) than those of plants without *Azolla* (7.2 kg/tray).

Table 2: Response of barley fresh and dry weight to *Azolla* addition under hydroponic system over replicates.

Treatments	Fresh Weight (kg/tray)	Dry Weight (kg/tray)
Control	7.2bc	1.5b
Treated	11.8a	2.9a

T-test in groups and LSD at p= 0.05 level of significance.

The results in Table 3 revealed the percentages of N, P, K and protein content under treated and untreated *Azolla* trays. Adding low surface *Azolla*, gave significantly higher N, P, K and protein content than untreated trays (0.98, 0.82, 0.70 and 28.7%, respectively) than those without *Azolla* (0.46, 0.11, 0.29 and 20.5 %, respectively).

Table 3: Response of barley chemical composition to *Azolla* addition under hydroponic system over replicates.

Treatments	N %	P %	K %	Protein content %
Control	0.46b	0.11 b	0.29 b	20.5 b
Treated	0.98a	0.82 a	0.70 a	28.7 a

T-test in groups and LSD at p= 0.05 level of significance.

Results in Table 4 showed disease incidence for treated plants with *Azolla* , which gave 0.0 % as compared with plants without *Azolla* (30 %) (Fig. 7).

Table 4: Response of barley disease incidence to *Azolla* addition under hydroponic system over replicates.

Treatments	Total No. plants	No. Infected plants	disease incidence %
Control	10.0	2.6 a	26.0 a
Treated	10.0	0.0 d	0.0 b

T-test in groups and LSD at p= 0.05 level of significance.

Fig. 7: Fodder barley free from root rots fungi with Azolla low

The results of this study showed using *Azolla* in cultivation the barley green fodder leads to plant height were higher 24 cm and also the protein content of barley were very high percentage 28.7%, However the fresh weight also high 11.8 kg/tray because *Azolla* have high nutrient content. The ability of nitrogen fixation of *Azolla* is due to the presence of the symbiotic cyanobacterium *Anabaena* that occurs in the dorsal leaf cavities of the fronds (**Peters and Meeks, 1989**). The symbiont is able to meet the entire nitrogen requirement of the association. Calvin cycle operates in both the partners and the primary end product of photosynthesis is sucrose (**Van Hove, 1989**). A strong interaction exists between nitrogen fixation and the photosynthesis and the source of ATP and NADPH is photosynthesis. The capacity of *Azolla* to fix nitrogen in the field has been estimated to be 1.1 kg N ha^{-1} day^{-1} (**Lumpkin and Plucknett, 1980**).

Because of the high nutrient content *Azolla* can be used as fodder for cattle and fish as well as poultry feed. It has Proteins, Vitamins, Calcium, Phosphorus, Iron, Copper, Magnesium, Beta carotene and Amino acids. It is observed that aquatic plant species especially *Azolla* offer a great potential as a source of protein for animals due to ease of cultivation, productivity and nutritive value (**Prabha and Kumar, 2010**). Poultry fed on *Azolla* show significant increase in the body weights and consequently have resulted in an increase in the net return (**Rai *et al.,* 2012**).

Seed soaking leads to the activation of enzymes and solubilisation and digestion of starch stored in the endosperm to simple sugars of the starch stored in the endosperm to simple sugars. This provides substrate for the young developing plant for metabolic activities. These substrates are respired to produce energy, giving off carbon dioxide and water. This loss of carbon dioxide leads to a loss in dry matter. These results were in accordance with those of **(Morgan *et al.*, 1992)**, who reported a significant difference in wet weight (WW) and dry weight (DW) of the hydroponic fodder. The height of barley sprouts ranged from 10 cm for Giza 127 to about 6.2 cm obtained by Giza 123. **Al-Hashmi (2008)** obtained similar values regarding to the sprouts height of hydroponic barley. However, the average sprout heights of barely cultivars showed significant differences among them. Fresh sprout weight: seeds weight ratio of different cultivars illustrated in the results indicated that the highest significantly value of fresh sprout: seeds weight ratio (6.7) was recorded by Giza 127 compared to the other five cultivars. The fresh sprout weight: seeds weight ratio ranged from 6.7 to 4.4 (Kg/Kg). Similar results were obtained by **Al-Hashmi (2008**) and **Al-Karaki, (2010)** they reported that the ratio reached up to 8 times in barley sprouts produced via intensive hydroponic system but under full control system.

The crude protein obtained in this study was comparable with those reported by **(Al-Ajmi *et al.,* 2009)** who found about 14 percent of crude protein in hydroponically barley green fodder. **(Morgan *et al.*, 1992)** reported that crude protein content was increased from 10.8 at day 4 to 14.9 percent at day 8 in hydroponically barley fodder that were in accordance with our findings. **(Sneath and McIntosh, 2003)** evaluated the composition of sprouted barley and reported that the crude protein ranged from 11.38 to 24 percent. However, protein content may be influenced as a result of the nitrogen supplementation and other nutrients changes in sprouting grains. **(Morgan *et al.,* 1992)**. Also, **(Peer and Leeson, 1985)** reported that protein content of green fodder is similar to barley grain, where the crude protein was higher in the green barley because of the relative decrease of other components.

Naik *et al.* (2015) estimated that sprouts are rich source of anti-oxidants in the form of b-carotene, vitamin-C, E and related trace minerals such as Se and Zn. As sprouted grains (hydroponics fodder) are rich in enzymes and enzyme-rich feeds are generally alkaline in nature, therefore, feeding of the hydroponics fodder improves the animals' productivity by developing a stronger immune system due to neutralization of the acidic conditions.

2. CONCLUSIONS

From results of this study, it can be concluded that the barley crops showed better fodder production by using Azolla under hydroponic conditions. And free from root rot fungi. However, barley crop is considered the best choice that can be used for production of hydroponic green fodder.

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