



A NEW WAY TO ELIMINATE THE ROOT ROT FUNGI IN GREEN FODDER UNDER THE HYDROPONIC CONDITIONS

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ABSTRACT

This study was done to indicate the activity of secondary metabolites of cyanophyta algal extracts for *Anabaena Azolla* which live in *Azolla* plant in Egypt and to study the effective of secondary metabolites in *An.Azolla* extracts (cold water and methanol extracts) to inhibition species of fungi (*Fusarium oxysporum*, *penicillium expansum*, *Aspergillus niger* and *candida albicans*) also used of HPLC analysis to detected some of active secondary metabolites was done only for methanol extracts also to preliminary statements that used of two extracts of blue green alga *Anabaena Azolla*. The role of *An.Azolla* extracts to inhibition growth of these fungi was detected through measured diameter of inhibition zone diameters were, 16.58, 11.42, 11.0 & 11.0 mm in case of *Fusarium oxysporum*, *Penicillium expansum*, *Aspergillus niger* and *candida albicans* respectively, but aqueous extract give inhibition zone were 18.15, 14.13, 14.0 & 13.0mm respectively. and the results showed these extracts had high correlation ratio and fungi shows high sensitivity to these extract. The mode of mechanism action to inhibition was on fungi cell wall by inhibition active enzymes, excelled treatment for cold water extract . The most important active compounds had been detected in HPLC were phenolic compounds and flavonoids compounds these all compounds detected specially in methanol extract of *An .Azolla*. 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 protein content and the percentages of N, P, K by 28.7% and 0.98, 0.82, 0.70 %, respectively, as compared to no addition of *Azolla*. Zero 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 and free from root rot fungi.

Keywords: *Hydroponic barley, root rot fungi, Anabaena Azolla, inhibition growth, Secondary compounds, HPLC. Antifungal . Bioactivity. Extracts*

أجريت هذه الدراسة للإشارة إلى نشاط المركبات الثانوية لمستخلصات الازولا أنابيينا التي تعيش في نبات الأزولا في مصر ودراسة المركبات الثانوية الفعالة في مستخلصات الازولا أنابيينا (الماء البارد ومستخلصات الميثانول) لتثبيت أنواع الفطريات (*Fusarium oxysporum* ، *penicillium expansum* ، *Aspergillus niger* و *Candida albicans*) كما استخدم تحليل HPLC للكشف عن بعض المركبات الثانوية النشطة كانت فقط لمستخلصات الميثانول وأيضًا إلى مركبات أولية استخدمت من مستخلصين من الطحالب الخضراء الزرقاء الازولا أنابيينا تم اكتشاف دور مستخلصات الازولا أنابيينا في تثبيط نمو هذه الفطريات من خلال القطر المقاس ومنطقة النسبة المئوية للتثبيت كانت الأقطار ١٦,٥٨ و ١١,٤٢ و ١١,٠ و ١١,٠ مم في حالة *Fusarium oxysporum* و *Penicillium expansum* و *Aspergillus niger* و *candida albicans* على التوالي ، لكن منطقة تثبيط إعطاء المستخلص المائي كانت ١٨,١٥ و ١٤,١٣ و ١٤,٠ و ١٣,٠ ملم على التوالي. والنتائج أظهرت أن هذه المستخلصات لديها نسبة ارتباط عالية وتظهر الفطريات حساسية عالية لهذه المستخلصات. كان أسلوب عمل الآلية لمنع تثبيط جدار الخلية الفطرية عن طريق الإنزيمات النشطة المثبطة ، المعالجة الممتازة لاستخراج الماء البارد. أهم المركبات النشطة التي تم اكتشافها في HPLC كانت المركبات الفينولية ومركبات الفلافونويد هذه المركبات التي اكتشفت عادة في مستخلص الميثانول من الازولا أنابيينا.

هدفت هذه الدراسة لتقييم استجابة إنتاج العلف الأخضر من الشعير والتركيب الكيميائي بإضافة الازولا لبيئة النمو تحت ظروف الزراعة المائية ودور ذلك في تثبيط فطريات العفن الجذرية تحت ظروف الزراعة المائية. تم إجراء التجربة تحت ظروف درجة حرارة الغرفة والإضاءة الاصطناعية في غرفة نمو مختبر الزراعة بدون تربة (المائية) ، جامعة مدينة السادات ، مصر. وأظهرت النتائج أن العلف الأخضر يمكن إنتاجه في ٨ أيام من الزراعة إلى الحصاد باستخدام تقنية الزراعة المائية. تم تسجيل أعلى قيم للمحصول الأخضر الطازج للمحاصيل ، وتمت معالجة الشعير باستخدام الازولا ١١,٨ كجم / صينية ، مقارنة بالنباتات التي لا تحتوي على الازولا ٧,٢ كجم / صينية أظهرت النتائج أن النسب المئوية لمحتوى N ، P ، K والبروتين في حالة النباتات المعالجة بـ الازولا ، كانت أعلى بشكل ملحوظ ٠,٩٨ ، ٠,٨٢ ، ٠,٧٠ ، و ٢٨,٧٪ على التوالي من تلك التي لا تمتلك أزولا. أظهرت النتائج عدم حدوث المرض في حالة النباتات المعالجة مع الازولا كانت (٠,٠) ٪ مقارنة مع النباتات دون الازولا ٢٦٪ يمكن أن نستخلص من هذه الدراسة أن محصول الشعير يمكن اعتباره الاختيار الأفضل لإنتاج الأعلاف الخضراء المائية باستخدام الازولا بها نسبة عالية من البروتين وخالية من فطريات أعفان الجنور.

INTRODUCTION

Anabaena Azolla is one of cyanophyta type which is contain of heterocyst and gas vacuole (Rippka, et al., 1979) *Anabaena* can produce toxins in fresh aquatic water ecology and the bloom of *An. Azolla* lead to produce alkaloids neurotoxins anatoxin (Sivonen , and Jones, 1999) show that *Anabaena* can produce three types of toxins like anatoxins(s) which is organophosphate toxins, carbamate toxins and saxitoxins (LD₅₀ is 10-30 mg/kg) (WHO, 2003). *Anabaena* also produce microcystin-LR which is type of hepato toxins and there (LD₅₀ is 40-70 mg/kg) and produce cylindrospermopsin which mechanism is to inhibition of Protein synthesis of fungi (Griffiths and Saker, 2003). *Anabaena* produce these toxins as secondary compounds that used to inhibition growth of pathogenic fungi and the unsaturated fatty acids consider important source to produce toxins because its contain of toxic chemical properties and these secondary compounds contracts with hormonal and toxically effects against microbes (Moe, 1997). This algae can produce lipopolysaccharides endotoxins inhibition of fungi (Blahova, et al., 2013) because of this algae can produce many types of toxins and for this reason we can used *Anabaena* extracts as antibiotics against *Fusarium oxysporum* and *Penecillium expansum* (Yousif, 2014). *An. Azolla* also used in biological control to inhibition of undesirable microbes because its contain many benefits with estats against fungi and this way is more safety for ecology by compare with synthesis fungal pesticides (Salman, 2015) .

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; Chung *et al.*, 1989) with health beneficial effects on animals (Boue *et al.*, 2003).

Azolla spp. are small free-floating aquatic pteridophytes, belonging to the family *Azollaceae*, with a worldwide distribution on tropical, subtropical and temperate regions. This fern is unique as it has an everlasting symbiosis with the nitrogen fixing cyanobacterium *Anabaena Azolla* inhabiting cavities in the *Azolla* dorsal lobes. This fern, although considered an invasive species, can be used as biofertilizer, in the phytoremediation of domestic and industrial wastewater and as animal or human food (Wagner 1997; Carrapiço 2010).

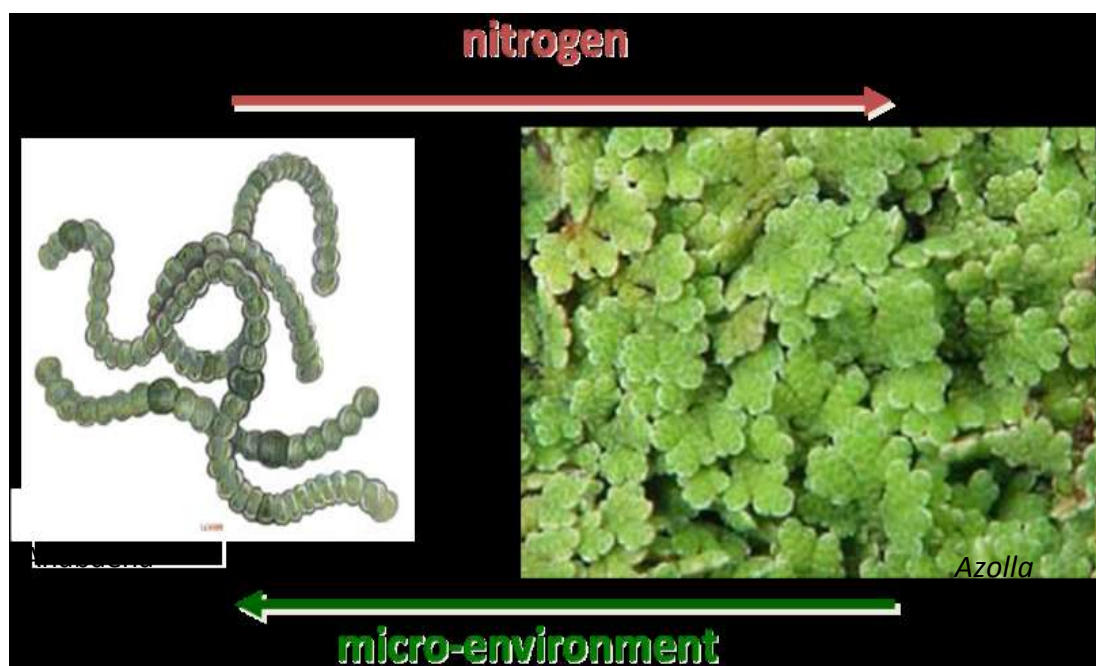


Fig. 1: *Azolla-Anabaena* system to fix atmospheric nitrogen

The aim of the present research was to evaluate the biological activities of organic and aqueous extracts from six *Azolla* species against root rot fungi.

1. MATERIALS AND METHODS

A) VITRO EXPERIMENTS:

1.1. SAMPLE COLLECTION AND IDENTIFICATION:

Azolla species were obtained from Agriculture Research Center Gizza, Egypt and were cultured in Hoagland medium (H-40), pH 6.1–6.2, with controlled temperature, photoperiod and light intensity (Pereira and Carrapiço 2009). The collected biomass was washed in water, stored at -80°C , lyophilized and weighted.

1.2. EXTRACTION OF AQUEOUS AND ORGANIC COMPOUNDS

The lyophilized biomass of *Azolla* was ground in a mill and extracted twice with dichloromethane: methanol (2:1, v/v) for 3 h, vacuum filtered through paper filter (Whatmann N° 1) and cheese cloth and evaporated until dryness in a rotary evaporator thus yielding the organic fraction which was weighted and stored at -80°C .

To obtain the aqueous fraction, the organic solvent extracted biomass was extracted twice with ultrapure water for 2 h, sonicated with an ultrasound probe at 20 kHz for 7 min., filtered through a cheese cloth and paper filter (Whatmann N° 1), centrifuged at 4542 rpm for 30 min. at room temperature. The supernatant was frozen, lyophilized until dryness, weighted and stored at

-80 °C. Prior to the bioassays, all extracts were resuspended in DMSO to obtain a stock concentration of 100 mg/ml, except for the organic extract and stored at -20 °C.

1.3. DETERMINATION OF ANTIMICROBIAL ACTIVITY

The obtained extract was dissolved in Dimethyl sulfoxide (DMSO).. Both bacterial and fungi microbes were grown on nutrient agar medium. The fungal strain was on other hand grown on PDA medium. The culture of each microorganism was diluted by sterile distilled water to 10^7 to 10^8 CFU/ml. The inoculated agar plates were incubated in the upright position for 24 hrs (bacteria) at 37° C for bacteria and 48h at 30°C for fungi and yeast. After incubation, the diameter of inhibition zones were measured against a wide range of test microorganisms comprising: Gram+ve bacteria; *Staphylococcus aureus* ATCC6538-P), Gram -ve bacteria *Pseudomonas areuginosa* ATCC 27853), yeasts (*Candida albicans* ATCC10231 and the fungus *Aspergillus niger* NRRLA-326. All test microbes were obtained from the culture collection center, Microbial Chemistry Department, National Research Center, Egypt and isolates *fusarium oxysporum*, *penicillium expansum* obtained from plant pathology lab, faculty of agriculture Mansoura university.

1.4. HPLC ANALYSIS:

The chemical composition of *Azolla* extract were performed in International Research Center, Gizza, Egypt (central labs unit) for detected phenolic, flavonoids and other active compounds.

1.5. ULTRASTRUCTURE STUDIES:

Method of specimen preparation for transmission electron microscopy

The method described here is an example for fixation, contrastation and embedding only . For optimal results especially the fixation needs to be adapted to the investigated tissue . It is of major importance that the fixing agent is able to completely. Penetrate the specimen as soon as possible. For this reason only very small pieces of tissue (~ 1 mm³) should be fixed if no perfusion fixation (fixative is directly infused into a larger vessel of a deeply anaesthized animal) should be possible. The preparation should be quick enough, i.e. 5 minutes after tissue does no longer receive oxygen, and it began to show first signs of degeneration of ultra-structures. Perfusion or immersion fixation of the tissue using a modified **Karnovsky (1965)** solution : 2.5 %buffered glutaraldehyde + 2 % paraformaldehyde in 0.1 M sodium phosphate buffer pH 7.4 , leave tissue overnight at 4° C , wash 3 x 15 minutes (min.) in 0.1 M sodium phosphate buffer + 0.1 M Sucrose , postfix 90 min. in 2 % sodium phosphate buffered osmium tetroxide pH 7.4 , wash 3 x 15 min in 0.1 M sodium p, after drying for ~15 min sections may be investigated in a transmission electron microscope . Ultrathin sections were observed at 160 kV using a JEOL JEM -2100 at EM Unit, Mansoura University, Egypt.

B) VIVO EXPERIMENTS:

This study was conducted under room temperature 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.

C) 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 (9 days), depending on crop variety and growth conditions (**Al-Karaki and Al-Momani, 2010**). The horizontal area occupied by each unit of the system was about 2 m² 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 58 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%.

1.6. 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.

1.7. 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.

1.8. 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 & Al Hasim, 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.



Fig. 3: *Azolla* culture spread at low surface

FORAGE YIELD:

The experiment was terminated after 8 days from seeding, where the fodder biomass was ready for harvest (Fig 4). 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 rot fungi. 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 4: Green fodder of barley crop ready for harvest.

1.9. 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 ($P \leq 0.05$).

2. RESULTS

2.1. ANTIMICROBIAL ACTIVITY:

In the present study, the antifungal activity of *Azolla* give higher inhibition zone more than methanolic extract. Data in table (1 & 2) and Fig (5) indicate that methanolic extract of *Azolla* showed inhibitory action against tested isolates, where inhibition zone diameters were, 16.58, 11.42, 11.0mm in case of *Fusarium oxysporum*, *Penicillium expansum*, *Aspergillus niger* and *candida albicans* respectively, but aqueous extract give inhibition zone were 18.15, 14.13, 12.0 & 13.0mm respectively. The bacterial isolate *pseudomonas areuginosa* was sensitive to *Azolla* aqueous extract, the inhibition zone was 13.0mm and 10.0 mm by methanolic extract, *staphylococcus aurous* have no inhibition zone.

Table (1) The effect of *Azolla* extracts on growth of tested fungi

concentration mg/ml	Inhibition zone (mm)							
	<i>F. oxysporum</i>		<i>Pen.expansum</i>		<i>Aspergillum niger</i>		<i>Candida albinos</i>	
	Cold water	methanol	Cold water	methanol	Cold water	methanol	Cold water	methanol
60	18.15	16.58	14.13	11.42	14.0	11.0	13.0	11.0

Table (3) The effect of *Azolla* extracts on growth of tested bacteria

concentration mg/ml	Inhibition zone (mm)			
	<i>pseudomonas areuginosa</i>		<i>staphylococcus aurous</i>	
	Cold water	methanol	Cold water	methanol
60	13.0	10.0	-	-

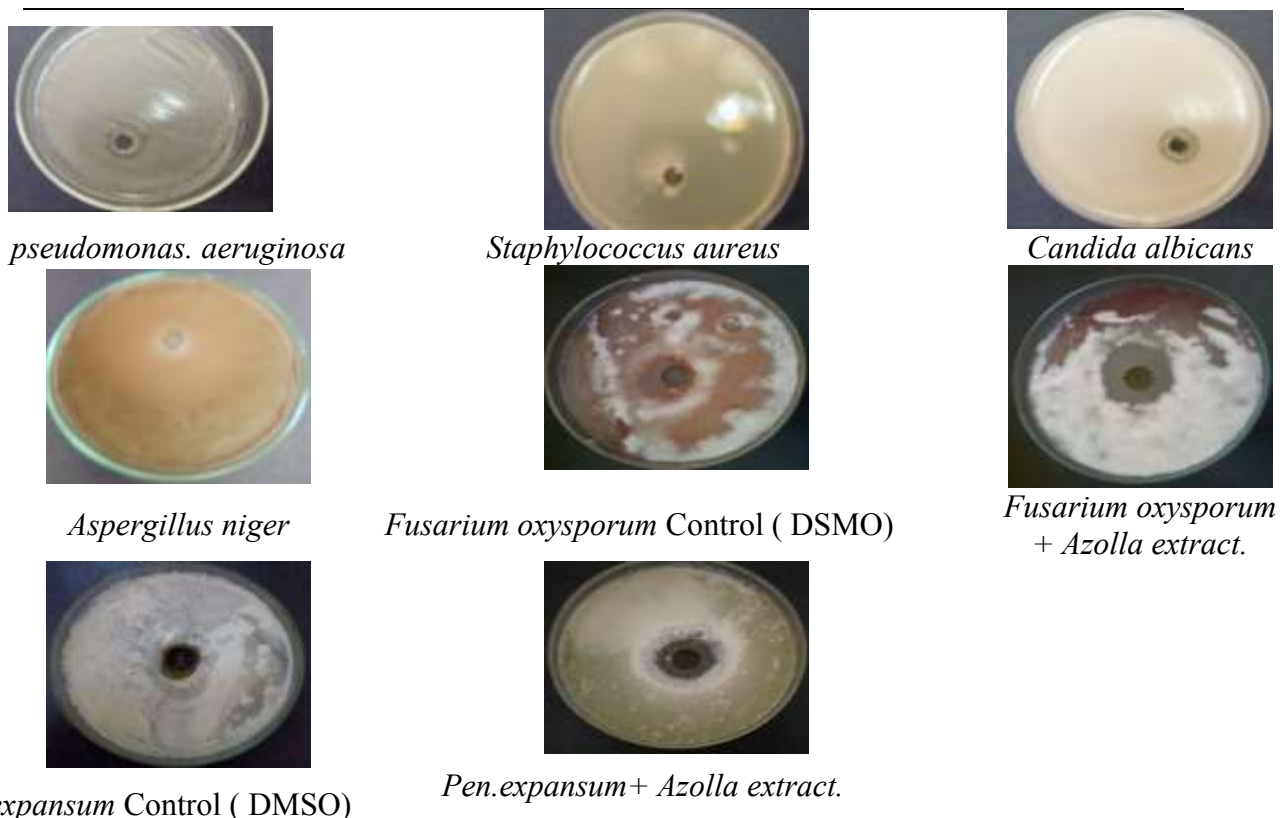


Fig (5): The effect of *Azolla* extracts on growth of tested fungi and bacteria

2.2. HPLC ANALYSIS:

The chemical composition of *Azolla* extract was carried out using Hplc analysis in central labes, National Research Center, Egypt. Table (3) and Fig (6&7) showed the extract of *Azolla* contain phenolic compounds such as p-hydroxybenzoic acid and flavonoids compounds such as caffeic acid.

Table (3): HPLC analysis of *Azolla* extract

Compound	Concentration (ug/g)
Gallic acid	ND
Protocatechuic acid	80.06
P-hydroxybenzoic acid	48.60
Gentisic acid	ND
Cateachin	404.06
Cholorgenic acid	ND
Caffeic acid	277.27
Syringic acid	510.41
Vanillic acid	28.69
Ferulic acid	279.23
Sinapic acid	108.43
P-coumaric acid	ND
Rutin	ND
Apigenin-7-glucoside	590.51
Rosmarinic acid	5553.41
Cinnamic acid	ND
Qurecetin	ND
Apigenin	ND
Daempferol	ND
Chrysin	ND

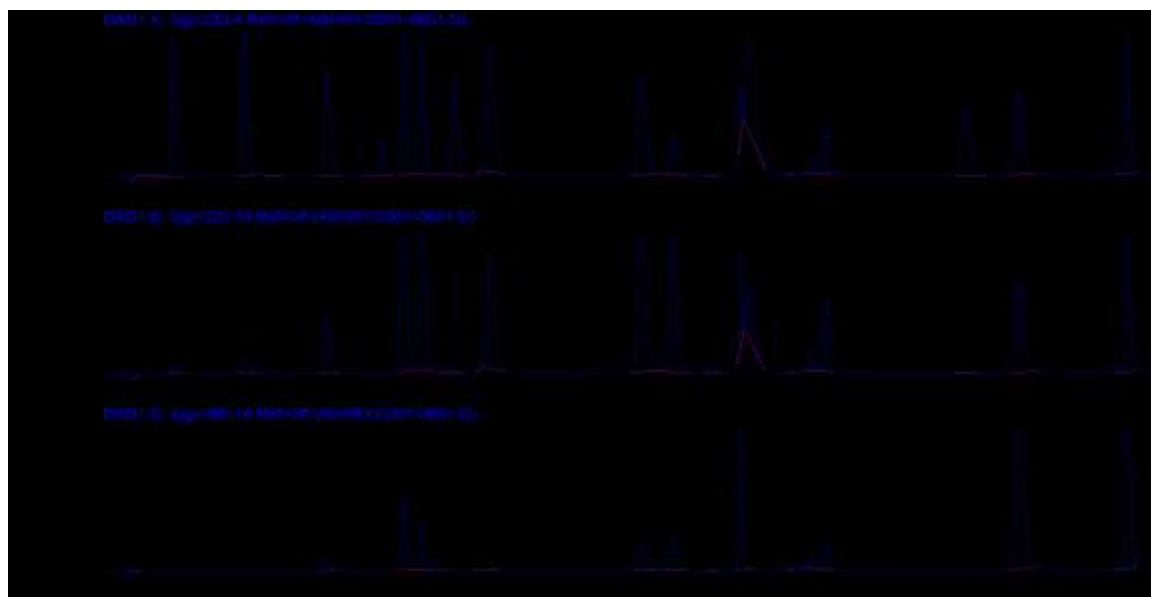


Fig (6). Chromatogram of HPLC analysis of *Azolla* extract (standard)

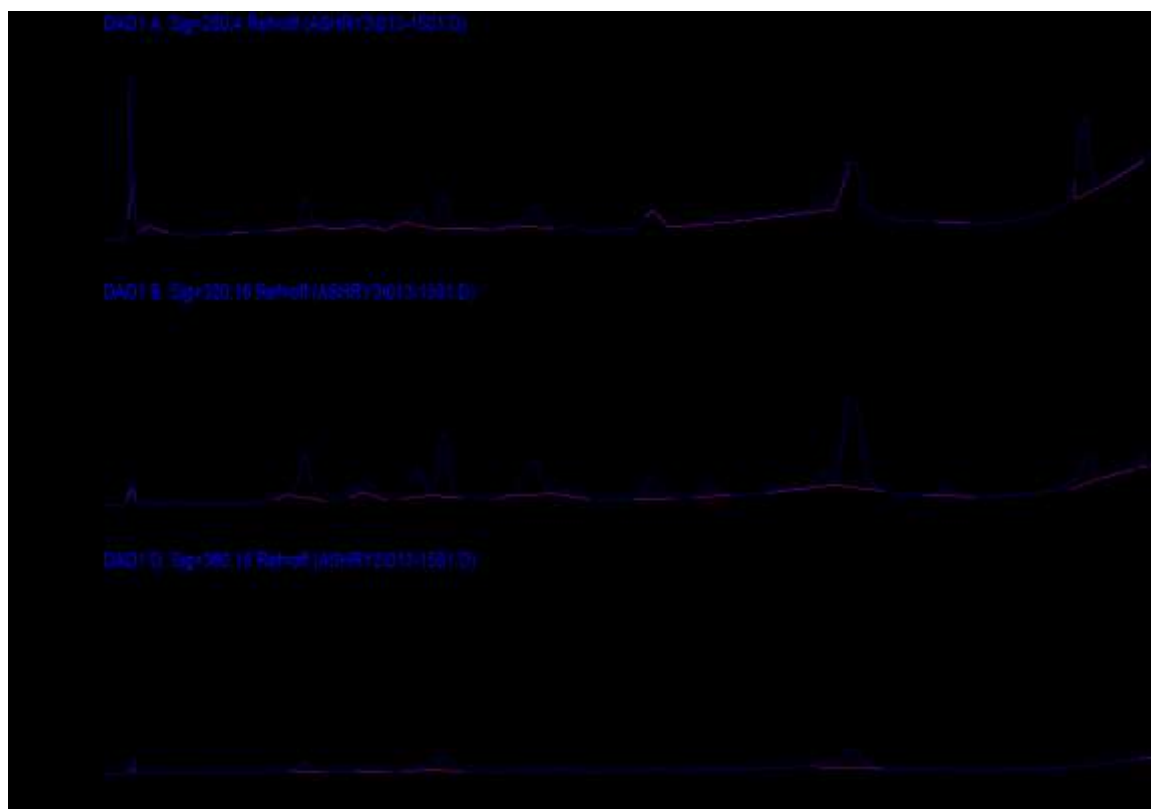


Fig (7). Chromatogram of HPLC analysis of *Azolla* extract (sample)

2.3. ULTRASTRUCTURE STUDIES:

The ultrastructure studies showing the effect of *Azolla* – anabaena extracts on *Fusarium oxysporum* cell Fig (8).

Transmission electron micrographs showing the effect of plant extract of *Azolla* on *Fusarium oxysporum*.

A: *Fusarium oxysporum* hypha grown on medium lacking plant extract of *Azolla*. A cell of *Fusarium oxysporum* surrounded by a thin wall (W) and the cytoplasm (Cy) contains numerous organelles such as mitochondria (M). Scale bar = 0.5 μ m (cited from **Baka et al., 2002**).

B, C and D: *Fusarium oxysporum* hypha grown on medium containing plant extract of *Azolla* at concentration of 0.4%. *Fusarium oxysporum* cell show a distorted wall (w), disintegration of cytoplasm (Cy). Note large lipid bodies (L), vacuoles (v) and not recognized plasma membrane. Scale bar=0.5 μ m.

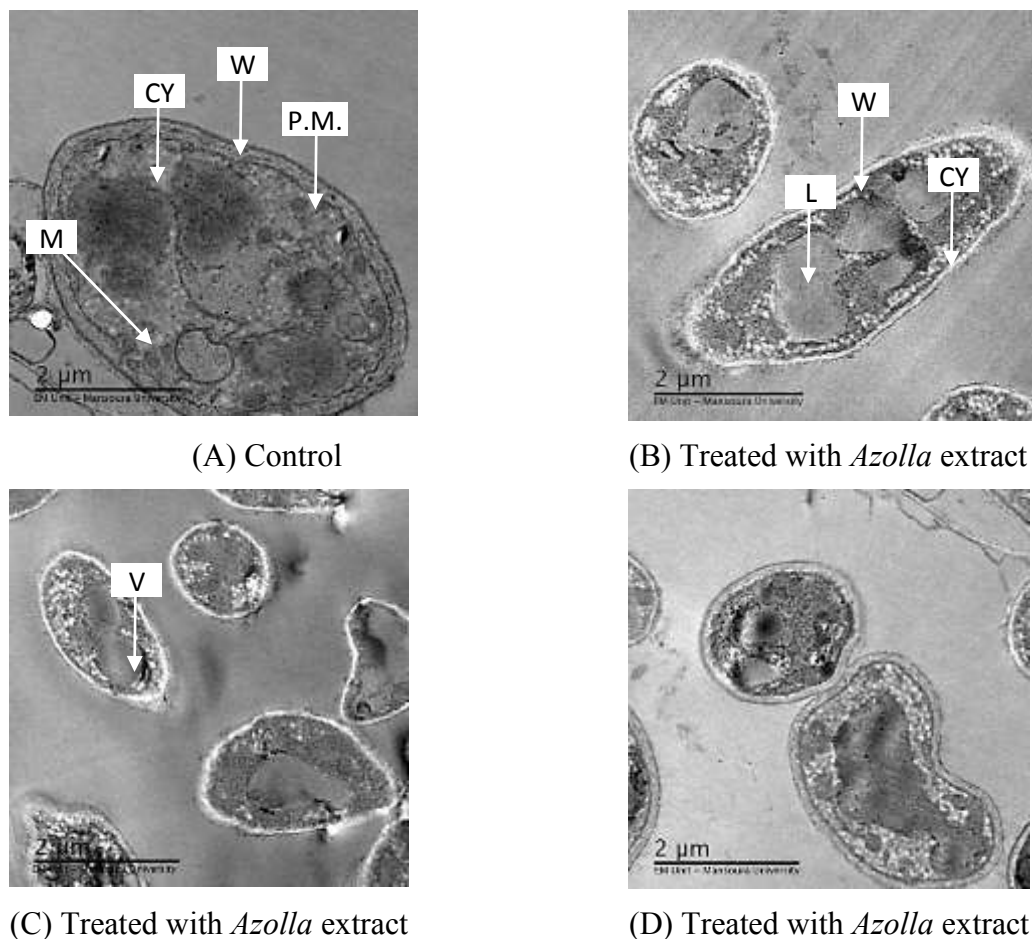


Fig (8): Transmission electron micrographs showing the effect of plant extract of *Azolla* on *Fusarium oxysporum*. (A) Control (B), (C) and (D) Treated with *Azolla* extract

The results of present study showed vegetative growth parameters and green forage production of barley after 8 days from planting (Table 4, 5, 6, 7 & Fig. 9)

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

Parameters	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 9: Green forage yield of barley with low surface layer of Azolla

The results in Table 5 revealed that the fresh weight of barley fodder per 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 5: Response of barley fresh and dry weight (kg/tray) to *Azolla* addition under hydroponic system over replicates.

Parameters	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 6 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, 28.7%, respectively) than those without *Azolla* (0.46, 0.11, 0.29, 20.5 %, respectively).

Table 6: Response of barley chemical composition to *Azolla* addition under hydroponic system over replicates.

Parameters	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 7 showed disease incidence for treated plants with *Azolla* , which gave 0.0 % as compared with plants without *Azolla* (30 %) (Fig. 10).

Table 7: Response of barley disease incidence to *Azolla* addition under hydroponic system over replicates.

Parameters	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. 10: Fodder barley free from root rots fungi with *Azolla* low surface

3. DISCUSSION

The results show that *An.Azolla* extracts had important role to inhibition of fungi also can produce active compounds like (Phenols, Flavonoids, Alkaloids, Terpens, Glycosides, Saponins) in natural aquatic ecology because the cold and hot water extracts show highly inhibition zone for *Fus.oxysporum* and *Pen.expansum* (Rossana, et al., 2006) . The Anabaena algae secrete active compounds to inhibition *Fus.oxysporum* and *Pen.expansum* because these extract maybe influenced with enzyme activity of these fungi and caused inhibition (Hellio, et al., 2001).

These fungi show high sensitive to these extracts specially for cold and hot water extracts this maybe because the methanol lead to reduce activity of some active compounds which present by measured the inhibition zone and inhibition percentage, phenolic compounds in these extracts can be used as antibiotics (Abd El-Aty, et al., 2014).

The mechanism of inhibition for these extracts was facultative for active function in fungi without any side effects (Salvador, et al., 2007) and inhibition of *Fus.oxysporum* and *Pen.expansum* may be caused by effect on Mycotoxins or on spores functions or by effect on cell wall that lead to inhibition fungal cell or by influence with cell permeability (Gupta, et al., 2001) or effect on *Fus.oxysporum* and *Pen.expansum* fatty acids by decreased of saturated and unsaturated fatty acids because these extract contain mixture of active compounds leads to inhibition of fungi(Persson, et al., 2011).

An.Azolla extracts may be acts in three mechanisms on these two type of fungi, the first one these extracts effect on ergosterol(azoles) (Sheehan, et al., 1999). The second mechanism was effect to inhibition of polyenes that contract with enzymes inside cell and the third mechanism was mainly effect on enzymes that made fungal cell wall and inhibition of cell membrane fatty acids synthesis then all of these lead to inhibition these fungi (Nozawa & Morita, 1989) .

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/per 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. , 199**) and (**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.

4. 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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