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Azolla Cultivation On Several Types Of Water Using Several Cheap Natural Fertilizers That Are Locally Available In Palestine

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Declaration

I certify that this thesis submitted for the degree of Master, is the result of my own research, except where otherwise acknowledged, and that this study (or any part of the same) has not been submitted for a higher degree to any other university or institute.

Signed :

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Date: 25/5/2022

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Thanks to **Almighty Allah**, who guides me and creates the reasons to finish this study, He gives me strength, determination, perseverance, and patience to complete this research. Peace and blessings be upon the best human being on earth our **Beloved Prophet Mohammed bin Abdullah**. All praise is to **Almighty Allah** for bestowing me with health, opportunity, patience and knowledge to complete this work .

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List of abbreviations

Symbol	Description
C_2H_4	Ethane
p.m	Post meridiem
NH_4	Ammonium
NO_3	Nitrate
NH_4NO_3	Ammonium nitrate
P_2O_5	Phosphorus pentoxide
Mo	Molybdenum
NaOH	Sodium hydroxide
CLLS	Closed-loop life support systems
CELSS	Controlled Environmental Life Support Systems
FCR	feed conversion ratio
DM	Dry matter
NDF	Neutral detergent fiber
ADF	Actin Depolymerizing Factor
P	Phosphorous
N	Nitrogen
K	Potassium
Ca	Calcium
Mg	Magnesium
Pb	Lead
Zn	Zinc
Ni	Nickel
Cd	Cadmium
$CuSO_4$	Copper sulfate
$Ca(H_2PO_4)_2$	Superphosphate
NaCl	Sodium chloride
COD	Chemical Oxygen Demand
C/N	Carbon Nitrogen ratio
Fe	Iron
Mn	Manganese
OM	Organic matter
CP	Crude protein
CF	Crude fiber
NFE	Nitrogen free extract

List of Units

Symbol	Description
C	Celsius
Cm	Centimeter
Kg	Kilogram
M	Meter
Mmol	Millimole
t/ha	ton per hectare
W/m^2	weight/m^2
mm	millimeter
Kcal	kilocalorie
ppm	parts per million
pH	Potential of hydrogen
Lux	luminous intensity
DT	Doubling time
TA	Total ash

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Abstract

The Azolla plant is one of the most important and famous plants that is worldwide cultivated for the production of animal feed. This is due to its cheap cost, rapid growth, and rich protein content, which ranges between (20-30)% of its dry weight. Also for its ease of cultivation, besides being environmentally friendly green fodder. Studies and scientific research dealing with this plant and its economic and agricultural importance to Palestinian farmers are so important in order to introduce Azolla cultivation in the West bank and Gaza strip. The plant is not yet known or widespread in the West Bank and Gaza Strip. Hence, it is necessary to conduct an objective scientific study on the ground that confirms and proves the possibility of cultivating this plant in the Palestinian environment using local organic fertilizers and on different types of water. Therefore, we planted several basins of Azolla plant in different environments in terms of temperature, light intensity, type of organic fertilizer and the amount of water used and compared with each other, to study the possibility of cultivating the plant in the Palestinian environment and to identify the most prominent environmental factors that affect its cultivation. This is the first and only study that investigates the possibility of cultivating Azolla in Palestine based on scientific investigations.

As it turned out that it is possible to grow the Azolla plant in Palestine with ease and outside the greenhouse for most months of the year, as well as inside it in all months and seasons. It was found that growth rates was higher during the summer by almost half. Also, the Azolla plant can be grown organically using natural organic fertilizers without using any chemical fertilizers. Vermicompost is the best ever among all the other organic fertilizers that can be used. This type of fertilizer does not need agricultural soil when used, unlike the rest of the organic fertilizers, which turned out to be unreliable alone for cultivation of the Azolla plant. Agricultural soil must be added with a height of 3 cm at least inside each basin when using these organic fertilizers. Azolla plant can be grown on many types of water, such as the water from fish farming ponds and the treated waste water secondary or tertiary treatment. The water of fish farming ponds was found the best among these types for cultivation Azolla. Azolla plant can be grown in a healthy manner at varying heights of water Ranging on average from 3 cm and above (it can be planted even in less than this

depth). The preferable basins depth during summer was found to be more than 20 cm. This depth was found better for control the temperature of water in the basin and climatic fluctuations between night and day. Within the Azolla basin (3-5) cm, experiments also showed that the Azolla plant multiplies in the Palestinian environment from (6-10) times within one month, i.e. an average of once every 4 days on average. The production per square meter of fresh green Azolla (within 4 days) and after drying in the shade for 5 hours ranged between 800 - 1000 gm. of fresh green Azolla, meaning that the monthly average production of a square meter of Azolla plant is equivalent to 6-7 kg/month. Therefore, the average biomass resulting from an area of one dunum during one month is approximately 6 tons.

The results also showed that the growth rate of the Azolla plant is close to the growth rate of the water lentil plant with a relative superiority in favor of the water lentil plant. It was also noted that the environment of the cultivated Azolla ponds could be improved by some additions. As an example for the fish water by adding agricultural soil, and daily stirring once or twice. Daily stirring of the water and soil of the organically grown Azolla ponds contributes to an increase in its growth rate. The intensity of light greatly affects the growth rate of the Azolla plant, its color and appearance. The growth rate of the Azolla planted in the shade (50% of the intensity of natural light) was greater than that directly exposed to sunlight. Sterilization of organic manure by the addition of copper sulphate at a concentration of (5 g/L)was found to play an important role in maintaining the safety of Azolla ponds from harmful algae, fungi and molds. It is also possible to fertilize and stir the water and soil of Azolla ponds built organically using different types of fish such as tilapia and catfish. With the need to take into account the numerical density (fish/square meter) because the goal of it is fertilization, not breeding, in order to maintain a balance between the needs of Azolla and the concentration of organic fertilizer. and Experiments have shown that the monthly water consumption rate by the Azolla plant is approximately equal to 120 m^3/dunum. And the palatability of farm animals was noted, different animals can eat Azolla.

زراعة الأزولا على عدة أنواع من المياه باستخدام العديد من الأسمدة الطبيعية الرخيصة والمتوفرة محليًا في فلسطين

اعداد: رائد حسن محمد عمرو

اشراف: أ. د معتز علي قطب

الملخص

يعتبر نبات الازولا أحد أهم و أشهر النباتات التي يتم زراعتها في العالم من أجل انتاج الأعلاف الحيوانية، و ذلك لرخص تكلفته، وسرعة نموه، ولغنى محتواه من البروتين والذي تتراوح نسبته بين (20 - 30)% من وزنه الجاف، وأيضا لسهولة زراعته، عدا عن كونه علف أخضر صديق للبيئة. ونظرا لقلة الدراسات والأبحاث العلمية التي تتناول هذا النبات واهميته الاقتصادية والزراعية للمزارعين فإنه لحد الأن غير منتشر في كل من الضفة الغربية و قطاع غزة بشكل عام. ومن هنا كان لا بد من عمل دراسة علمية موضوعية على أرض الواقع تؤكد وتثبت امكانية زراعة هذا النبات في البيئة الفلسطينية باستخدام أسمدة عضوية محلية وعلى أنواع مختلفة من المياه. و لذلك قمنا بزراعة عدة أحواض من نبات الازولا في بيئات مختلفة من حيث درجة الحرارة، كثافة الضوء، السماد العضوي، نوع المياه المستخدمة و المقارنة فيما بينها، لدراسة امكانية زراعة النبات في البيئة الفلسطينية و تحديد أبرز العوامل البيئية التي تؤثر في زراعته.

حيث تبين انه بالإمكان زراعة نبات الأزولا في فلسطين بكل سهولة و خارج البيت البلاستيكي معظم أشهر العام، وكذلك داخله جميع الأشهر والفصول لكن بمعدلات نمو تزداد خلال الصيف و تقل خلال الشتاء الى النصف تقريبا، كما يمكن زراعة نبات الأزولا بشكل عضوي باستخدام الأسمدة العضوية الطبيعية بدون استخدام أي أسمدة كيماوية. و يعتبر سماد الفيرمي كمبوست الأفضل على الاطلاق من بين جميع الأسمدة العضوية الاخرى التي استخدمناها ولا يحتاج هذا النوع من الأسمدة لتربة زراعية عند استخدامه بعكس بقية أنواع الأسمدة العضوية والتي تبين بأنه لا يمكن الاعتماد عليها فقط في زراعة نبات الازولا بل يجب اضافة التربة الزراعية بارتفاع 3 سم على الاقل داخل كل حوض، كما يمكن زراعة نبات الازولا في العديد من انواع المياه، مثل مياه برك تربية الأسماك و المياه المعالجة معالجة ثانوية أو ثالثة، و يعتبر ماء برك تربية الأسماك الأفضل بين هذه الأنواع. و يمكن زراعة نبات الأزولا بشكل صحي على ارتفاعات متفاوتة من المياه تتراوح بالمعدل من 3 سم فما فوق (و يمكن زراعتها على أقل من هذا العمق) ويفضل أن يكون عمق الأحواض في فصل الصيف أكثر من 20 سم وذلك للتحكم بشكل أفضل في درجة مياه الحوض والتقلبات المناخية بين الليل والنهار، أما خلال فصل الشتاء فيكفي ان يكون ارتفاع المياه داخل حوض الأزولا (3-5) سم، كما بينت التجارب بأن نبات الأزولا يتضاعف في البيئة الفلسطينية من (6-10) مرات خلال شهر واحد، أي بمعدل مرة واحدة كل 4 أيام بالمتوسط، حيث كان معدل انتاج المتر المربع الواحد من حوض الازولا (خلال 4 أيام) وبعد تجفيفها في الظل لمدة 5 ساعات يتراوح ما بين 800 - 1000 غم من الازولا الخضراء الطازجة، أي أن

المتوسط الشهري لإنتاج المتر المربع من نبات الازولا يعادل 6 - 7 كغم / شهر، وبالتالي فأن معدل الكتلة الحيوية الناتجة عن مساحة دونم خلال شهر واحد تعادل 6 طن تقريبا.

كما بينت النتائج بان معدل نمو نبات الازولا قريب من معدل نمو نبات عدس الماء مع تفوق نسبي لصالح نبات عدس الماء، و لوحظ أيضا بأنه يمكن تحسين بيئة أحواض الازولا المزروعة على ماء السمك بإضافة تربة زراعية، كما يساهم التحريك اليومي بمعدل مرة أو مرتين لمياه و تربة أحواض الازولا المزروعة عضويا في زيادة معدل نموها، و لوحظ أيضا بأنه يمكن حماية أحواض الأزولا من الصقيع و البرد من خلال تغطيتها بأغطية بلاستيكية شفافة خلال الأيام والليالي الباردة، و بينت التجارب بأن كثافة الضوء تؤثر بشكل كبير على معدل نمو نبات الازولا و لونه و مظهره حيث كان معدل نمو الازولا المزروعة في الظل (50% من كثافة الضوء الطبيعي) أكبر من تلك المعرضة لأشعة الشمس بشكل مباشر، كما يساهم تعقيم السماد العضوي (الذي سيضاف لأحواض الازولا) بمركب كبريتات النحاس بتركز (5 غم/ لتر) دورا هاما في الحفاظ على سلامة أحواض الازولا من الطحالب والفطريات و الاعفان الضارة، كذلك يمكن تسميد وتحريك مياه وتربة أحواض الأزولا المبنية بشكل عضوي باستخدام أنواع مختلفة من الأسماك مثل سمك البلطي والسمك القرموطي مع ضرورة مراعاة الكثافة العددية (سمكة/ متر مربع) لأن الهدف منها يكون التسميد و ليس التربية و ذلك للحفاظ على التوازن بين احتياجات الازولا وتركيز السماد العضوي، كما بينت التجارب بأن معدل استهلاك المياه الشهري من قبل نبات الازولا يساوي تقريبا 120 م^3/ دونم، ولوحظ استساغة حيوانات المزرعة المختلفة لتناول نبات الازولا .

كلمات مفتاحية: ازولا بيناتا، معدل النمو، الكتلة الحيوية.

Chapter 1

Introduction

The livestock sector in Palestine faces many important and fundamental challenges that have made many farmers reluctance to raise livestock of all kinds, and one of the most important challenges and obstacles is the difficulty of providing animal feed at cheap and competitive prices, which greatly limits farmers' profits and reduces the feasibility of raising livestock in Palestine. Therefore, an urgent need arose to provide a new, safe, environmentally friendly, and easy source of animal feed in the Palestinian environment . On the other hand, the high prices of chemical fertilizers are among the most prominent and most important problems facing farmers working in the field of plant production. A realistic, easy, safe and environmentally friendly solution that solves the problem of the two sectors together, and one of the solutions proposed by many researchers around the world whose countries suffer from the same problem that we have was to resort to planting the Azolla plant, which can be used as animal feed due to the high protein content inside it and can also be used as natural fertilizer (Palestinian Water Authority, 2013).

On the other hand, we have a wasted water wealth represented in wastewater, which can be treated and used to cultivate the Azolla plant for later use as natural fertilizer, or water in fish breeding ponds whose water can be used excellently to grow the Azolla plant and provide animal feed at cheap prices and in easy and safe ways and environment friendly.

1.1 *Azolla pinnata* (Azollaceae – Mosquito ferns)

Azolla is a small free floating aquatic fern native to Asia, Africa, and America (figure 1.2). Azolla commonly known as mosquito fern, duckweed fern, fairy moss, and water fern. It grows in swamps, ditches, and even in lakes and rivers where the water is not turbulent (Kathirvelan, Banupriya et al. 2015). Azolla grow as a cluster of leaves, and the plant size range from 1 – 20.5 cm, some species can grow to a size of 15 cm (figure 1.1) (Wagner 1997) .

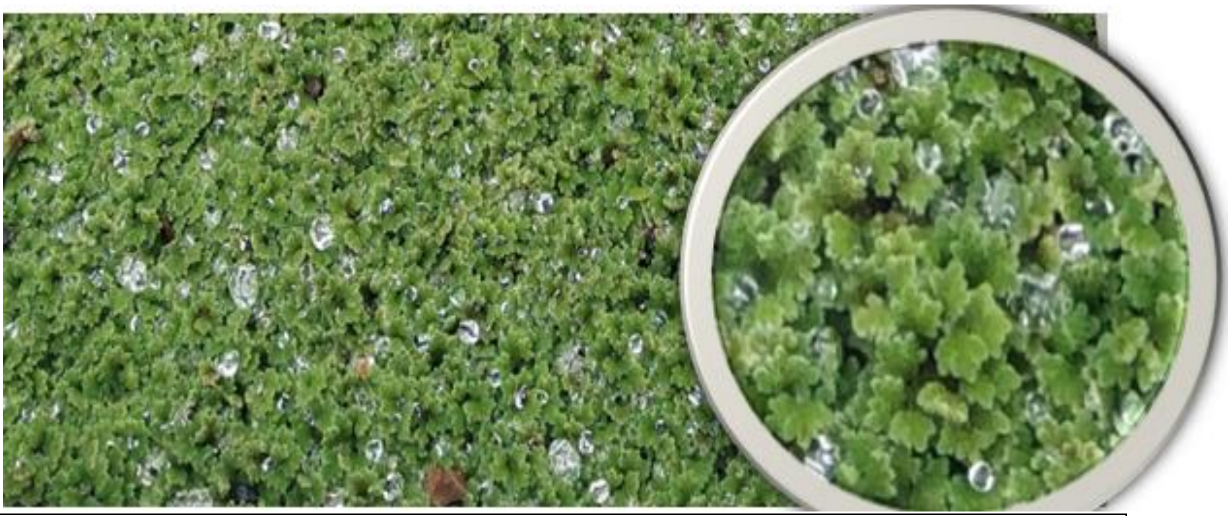

Figure 1.1: Azolla pinnata form.

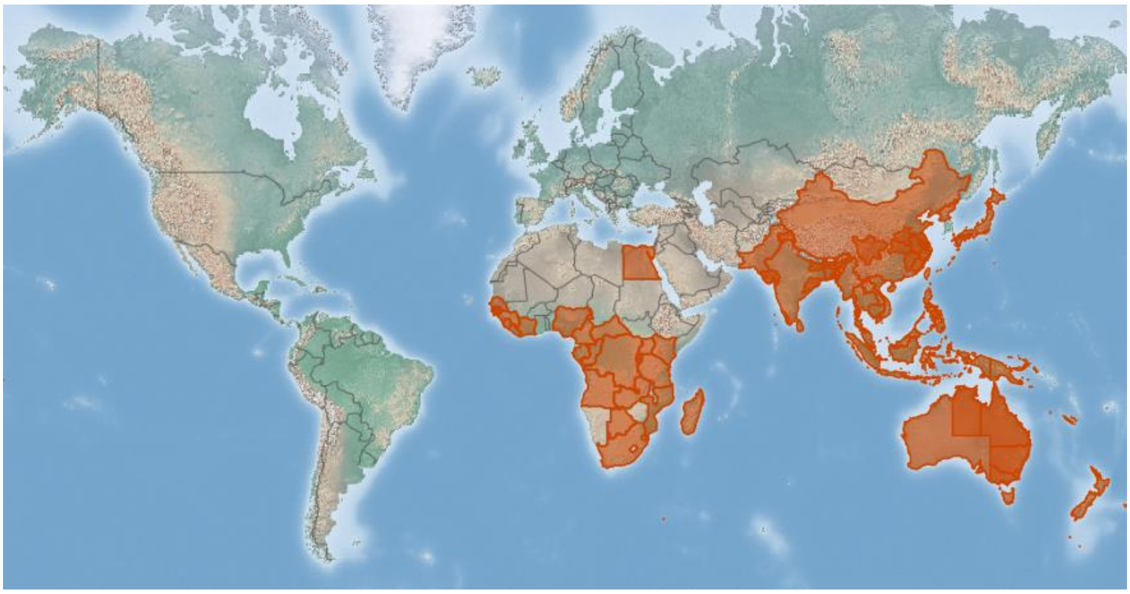

Figure 1.2: Map showing the distribution of the Azolla pinnata around the world. (CABI 2021)

1.1.1 Growth Rate of Azolla

Reducing the doubling time (DT) to three days or less will be very difficult to obtain a harvest with constant production, it may be possible to reduce the reproduction time of Azolla under industrial conditions Controlled or in laboratory conditions to become less than 30 hours (Mohamed 2005).

Sucrose is the primary end product in Azolla. 2% system Concentrations of glucose, fructose, sucrose or maltose in the nutrient solution increased the growth rate of

Azolla. Whereas, some sugars such as sucrose can increase Azolla yields by 183% over sugar-free control (Nickell 1961).

Hechler and Dawson (1995) found that the high density of the plant reduced the specific nitrogenase activity per unit biomass and per unit area. They found that the optimum plant density for nitrogenase activity in *Azolla caroliniana* was 50-100 g dry weight/m^2. It found that *Azolla pinnata* outperformed *Azolla filiculoides* when it gave higher growth of N, P and K contents in all of the tested growth periods. *Azolla pinnata* nitrogenase was also more active than *Azolla filiculoides*. They recommended *Azolla pinnata* for applications as a green fertilizer (El-Araby 1999).

1.1.2 Reproduction of Azolla

Azolla reproduces by vegetative and gender reproduction. Under natural conditions Azolla reproduces by vegetative propagation. Under certain circumstances genital formation is observed and a new generation of fertilized fetus is formed. Little is known about the state of spore formation and its environmental significance (Watanabe 1982).

1.1.2.1 Vegetative reproduction in Azolla

Vegetative propagation is the most common method of reproduction in nature and the agricultural application consists of multiplying by segmentation of the fronds. This occurs when roots or secondary branches form a separation layer at their bases and break off from the main rhizome. Root begins to appear from the base of the separation branch once the cell of the separation layer begins to mature. After separation from the parent plant. And new plants behave independently. The small plant also grows in size. It also grows more roots. It grows fully in 15-20 days (Lumpkin 1987). Azolla is a heterosporous plant, that is, it contains two types of spores that are produced on the same plant. Ovulation occurs most often during a specific period of the year, but not all species (Becking 1978)

At the first of sporulation ventral leaf lobe initial of a lateral branch, instead of forming a leaf lobe, the fern produces two sporocarps which are of two types, the microsporocarps and the megasporocarps. The microsporocarps, which are about 2 mm in diameter, each containing 32 or 64 spores which called microspores aggregated into 3-10 massulae, each produce 8-130 microsporangia (figure 1.3) (Ashton 1977).

The megasporocarps, which are about 0.5 mm in diameter produces a single megasporangium containing a single megaspore. At maturity, both the micro and megasporocarps dehisce. The microsporangia break and open, releasing microspores into the filamentous structures known as glochidia and sink to the bottom. After a period of stagnation each microspore germinates and grows into a prothallus that in turn produces ciliated, male gametes (anthropoids) (Moore 1969).

It found that spores were produced only by *Azolla pinnata*. Moreover, summer season with its high light intensity, high temperature and long day hour, represents the most suitable conditions for *Azolla pinnata* sporulation compared to those of other seasons. He also found that the lowest amount of sporulation was observed in winter (El-Shahat 1997).

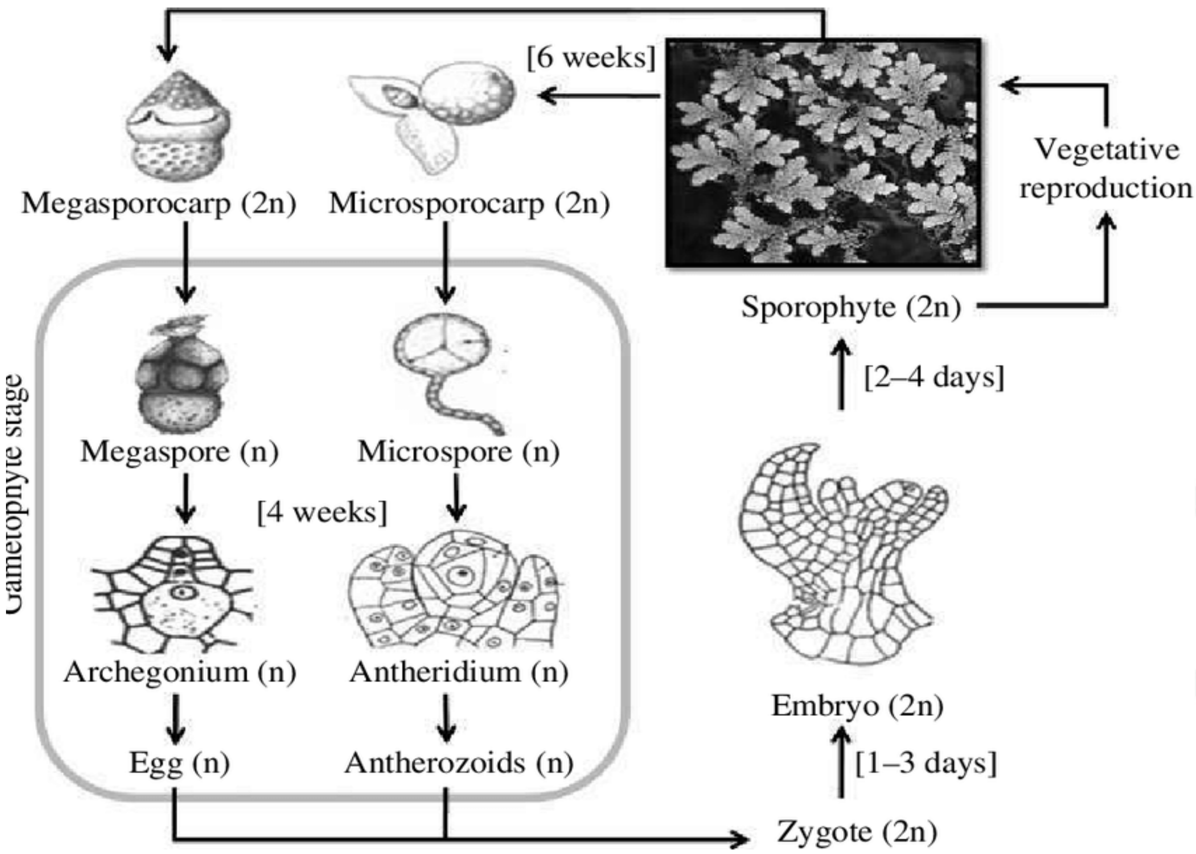

Figure 1.3. life cycle of Azolla.

1.1.3 Chemical composition of Azolla

The chemical composition of Azolla is another selective parameter that varies not only with species and environmental patterns but also with environmental conditions and stage of growth. They can be different aspects of the chemical composition considered. Depending on whether Azolla will be used as green fertilizer, food or other uses. Azolla plants have 94.96% moisture content (Liu 1979, Singh 1979, Van Hove 1989).

Chemical analysis showed that Azolla powder contains (% DM) 21.4 crude protein, 12.7 crude fiber, 2.7 ether extract, 16.2 ash and 47.0 carbohydrates. A total capacity of 2039 kcal was obtained. The concentrations of calcium, phosphorous, potassium, and magnesium were 1.16% 1.29%, 1.25% and 0.25% respectively, while sodium, manganese, iron, copper and zinc are 23.79 ppm, 174.42 ppm, 755.73 ppm, 16.74 ppm and 87.59 ppm respectively. The chemical grade index showed the potential of Azolla meal as a good source of protein. Leucine, lysine, arginine, and valine were the predominant essential amino acids while tryptophan and the sulfur-containing amino acids were deficient (Van Hove 1989).

In another study in which a sun-dried sample from *Azolla pinnata* was used for chemical composition analysis. The approximate composition and fiber fractions of cell wall components and mineral composition were estimated by standard methods. The approximate composition, cell wall components and trace minerals (copper, zinc, manganese, and iron) of sun-dried Azolla were quantified by standard methods. Dry matter, crude protein, organic matter, organic matter extract, total ash were 90.00 ± 0.77, 22.05 ± 0.72, 81.05 ± 0.44, 3.25 ± 0.76, and 18.94 ± 0.31. The content of NDF, ADF, hemicellulose, lignin, and cellulose was 48.25 ± 0.48, 37.14 ± 0.11, 11.11 ± 0.29, 8.07 ± 0.25, and 28.87 ± 0.64. It was zinc (ppm), copper (ppm), manganese (ppm), iron (ppm) and calcium (%) 30.02 ± 2.39, 26.29 ± 1.41, 348.17 ± 7.26, 533.12 ± 96.56 and 0.33 ± 0.03 (Liu 1979).

The nutritional composition of Azolla is affected by the time or age of the harvest, the method of drying and exposure to sunlight, it was found that sun dried Azolla has a higher nitrogen value than air dried (Alviar 1984, Van Hove 1989).

The total carbohydrate content of Azolla, which was exposed to different conditions of dark and light, did not show any significant differences. They demonstrated that *A. pinnata* had higher carbohydrate levels than *A. microphylla* and *A. filiculiodes* (Herzalla, Nagat et al. 2001.)

Table 1.1: Analysis of the components of the Azolla plant (Kumar, Dhuria et al. 2018).

Constituents	% DM
Dry matter (DM)	91.78
Organic matter (OM)	74.50
Crude protein (CP)	22.25
Crude fiber (CF)	11.19
Ether extract (EE)	2.45
Nitrogen free extract (NFE)	38.61
Total ash (TA)	25.50

1.2 Environmental factors that affect the growth rate of the Azolla plant

1.2.1 Temperature

The optimum temperature for Azolla Spp is between 18 and 28 ° C (Tuan and Thuyet 1979, El-Haddad, Mahmoud et al. 1988). But many species can survive very wide temperatures, such *as A. pinnata*, *A. mexicano*, and *A. Caroliniana*, although growth rates are low above 35 ° C and no species can survive long temperatures above 45 °C (Lumpkin 1987, Peters 1991).

El-Haddad, Mahmoud et al. (1988) suggested that the conditions in summer are the optimum climatic conditions for maximizing the growth of *A. pinnata*. The highest value of nitrogenase activity was obtained in August at the end of the third week of incubation, which was 13.8 μmol of C_2H_4/g dry weight at 1 pm in the case of *A. pinnata*. Peters (1991) reported that an Australian isolate of *A. pinnata* could survive at 55 ° C. Hechler and Dawson (1995) found that nitrogenase activity in *A. caroliniana* was very low in the early morning, and during mid-morning it rose sharply and reached at 12:00 - 3:00 pm maximum. Then in the evening it fell to a low level. Also, nitrogenase activity in *Azolla caroliniana* could be detected at a temperature between 5 and 40 °C, and it was relatively high between 15 and 35 °C.

Azolla tolerates a wide range of temperatures depending on its type and strain. Also, its response to monsoon summer temperature is not well understood. A research was conducted to study the effect of seasonal summer temperature on the productivity of green and dry biomass of Azolla. Green and dry biomass production was observed at a standard 6 to 13 weeks. The results showed that the maximum air temperature between 31.37 and 33.74 degrees Celsius and the minimum temperature between 22.81 and 23.34 degrees Celsius and the water temperature between 32.6 and 34.4 degrees Celsius boosted the productivity of Azolla biomass, while the Azolla colonies perished at the air temperature above 35.2 degrees Celsius (Tuan and Thuyet 1979).

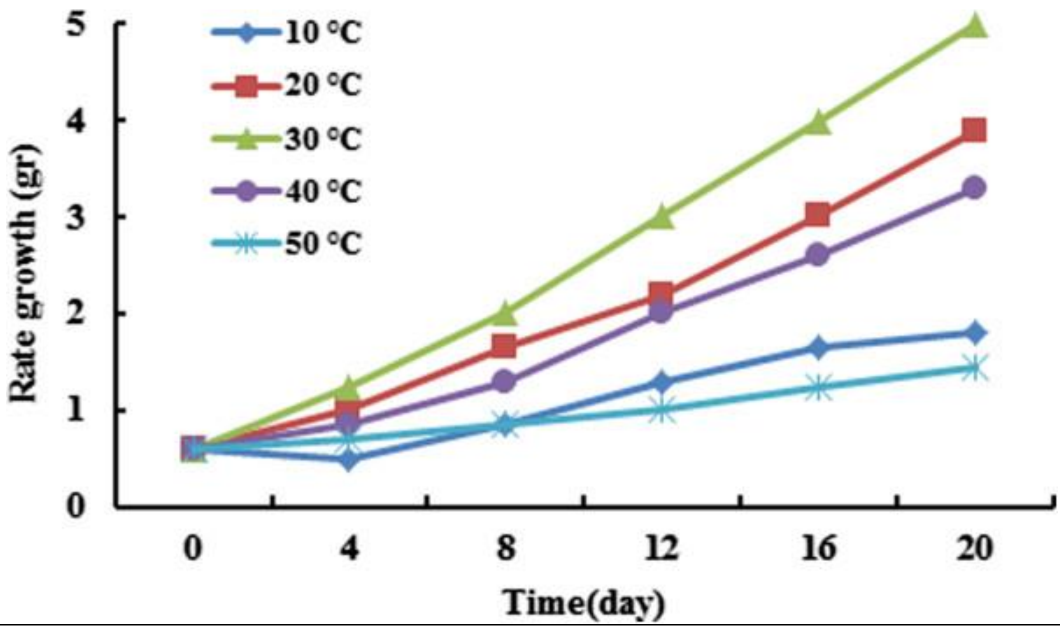

Figure 1.4 : The effect of temperature on Azolla growth rate, initial biomass = 0.9 g. (Zazouli, Mahdavi et al. 2014)

1.2.2 Humidity

To some extent, the increase in the biomass of Azolla depends on the humidity of the air. At a relative humidity of less than 60%, Azolla becomes brittle and dry (Bocchi and Malgioglio 2010).

To achieve the best growth rate, many researchers have found that the relative humidity should range between 70 and 75% (Forni, Chen et al. 2001, Costa, Santos et al. 2009, Sadeghi, Zarkami et al. 2012a). Sadeghi, Zarkami et al. (2012a) has showed that the spread of Azolla is weak when the air humidity in Anzali's wetland exceeds 80% . Average relative humidity was estimated to allow for Azolla growth 55-83% (Lumpkin and Bartholomew 1986). Based on the study of Biswas, Parveen et al. (2005) the humidity range was between 65-75%. However, according to the authors, for optimal growth of Azolla production and biomass, high temperatures, low water depths and high humidity may not be particularly good conditions during the dry season. When the weather is completely dry, the fern dies . Therefore, Azolla needs sufficient moisture to grow and reproduce successfully.

1.2.3 Light intensity

Scientific studies prove that light intensity has a profound effect on *Azolla pinnata R*. Brown's growth. The maximum fresh weight, dry weight and moisture content reached 80,000 lux, but at a light intensity of 50% the maximum area for the frond was reached. There was great variation in the color of the fronds under the different treatments. The texture of the fronds, root properties, and sporocarp production were also affected (Singh and Srivastava 1985).

In another study, *A. microphylla* and its symbionts and Azolla had the best sunlight intensity of 70%. Dynamic growth was recorded during the first week after fertilization as time is doubled. It is recommended that *A. microphylla* and its symbionts and Azolla are below 70% of sunlight intensity (Effendi and Pranata 2019).

The reason for this is that the light intensity influences the photosynthesis, growth, and nitrogen fixation activity of Azolla and its cohabitation, Sporulation is regulated by the interacting effects of light intensity, temperature ,photoperiod, and other factors such as pH, nitrogen and phosphate supply, When the intensity of the light is high and the amount of nutrients in the water is low, the Azolla turns red. During hot summers or cold winters, it also turns red or brownish-red under shady conditions, while it turns green in nutrient-rich conditions. With increased light, the growth rate and nitrogen fixation activity of Azolla changes. Some studies indicate that at pH 5, higher light intensity led to increased Azolla growth, while at pH 6 and 7, it impeded growth and high light intensity (above 90 clux) prevented N_2 fixation, while low light intensity or shading had a good effect On the growth and reproduction of Azolla.

light intensity less than 10-13 clux can reduce Azolla's nitrogen fixation. In field conditions, Azolla benefits from shade to grow. However, when the light intensity is less than 1.5 clux, Azolla biomass production decreases significantly. In conclusion, the optimum light intensity for the growth of Azolla is 15-18 clux, and their growth and photosynthesis are inhibited at higher intensities (Sadeghi, Zarkami et al. 2013).

1.2.4 Biological Factors

The presence of some macrophytes communities (such as Phragmites spp. and Typha spp.) can play an important role in overgrowth of Azolla in wetlands. Macrophytes occupy wetlands in the shallow part of the wetland. This provides an excellent opportunity for Azolla distribution because phragmite creates shelter and windbreaks for these species. (JICA 2005, Sadeghi, Zarkami et al. 2012a, Sadeghi, Zarkami et al. 2012b).

On the one hand, and on the other hand, some biological factors negatively affect the growth of Azolla. For example, some insects like *Diptera Lepidoptera* (larvae), Cephalopods and Snails. As it affects the growth of the Azolla plant through "grazing" on its biomass during its different growth stages. In addition to the use of chemical pesticides or herbicides while they are growing (McConnachie, De Wit et al. 2003). Thus, some or all of these biological agents can be used to control Azolla. Mechanical removal of some Azolla species (such as *A. filiculoides*) will also not be practical due to the very rapid rate of doubling of the surface area of Azolla (McConnachie, De Wit et al. 2003). Biological weed control such as Azolla can be a viable option, provided the target plant population is significantly reduced and no additional control methods are required (Ghosheh 2005). This indicates that biological weed control methods should be examined in depth.

Microbiological pathogens such as bacteria, fungi and viruses have been studied for biological control of *A. philoxeroides*, *A. filiculoides* and other aquatic plants in tropical areas these pathogens may affect the growth and reproduction of the species by reducing growth and fixing nitrogen in association (Barreto, Charudattan et al. 2000).

The forage weevil *Stenopelmus rufinasus* (Gyll.) Can be found in *A. caroliniana* and *A. filiculoides*, therefore among Azolla species. *A. filiculoides* have been a target of biological control programs (Gassmann, Cock et al. 2006). For example, in South Africa, *S. rufinasus* was imported from Florida and used as a biological control agent in 1997, the weevil was able to contain the population of Azolla for 3 years. Azolla was no longer considered a problem in South Africa after this period (Ghosheh 2005).

1.2.5 Growing seasons and day length

Another important climatic factor that regulates the production of aquatic plants is the length of the growing season and the length of the day (Serag, El-Hakeem et al. 2000). Biomass production and growth of Azolla also depend on the specific growing season. However, the growing seasons in Azolla are related to other factors such as nutrients, salinity, wind and pH. Also, a water body that has a high percentage of phosphorous and a neutral pH is better than a water body that has a low percentage of phosphorous and acidity. In general, the production of Azolla biomass is higher in summer than in other seasons (Speelman, van Kempen et al. 2009).

Any changes in weather conditions over the different seasons have an important effect on the growth of Azolla, because the change in weather conditions directly affects the air temperature, which is directly related to the water temperature (Ferdoushi, Haque et al. 2008). Janes (1998) studied the growth and survival of *A. filiculoides* in Britain where this plant is considered a dangerous weed. According to the author, the long-term survival of Azolla may be limited due to the winter death.

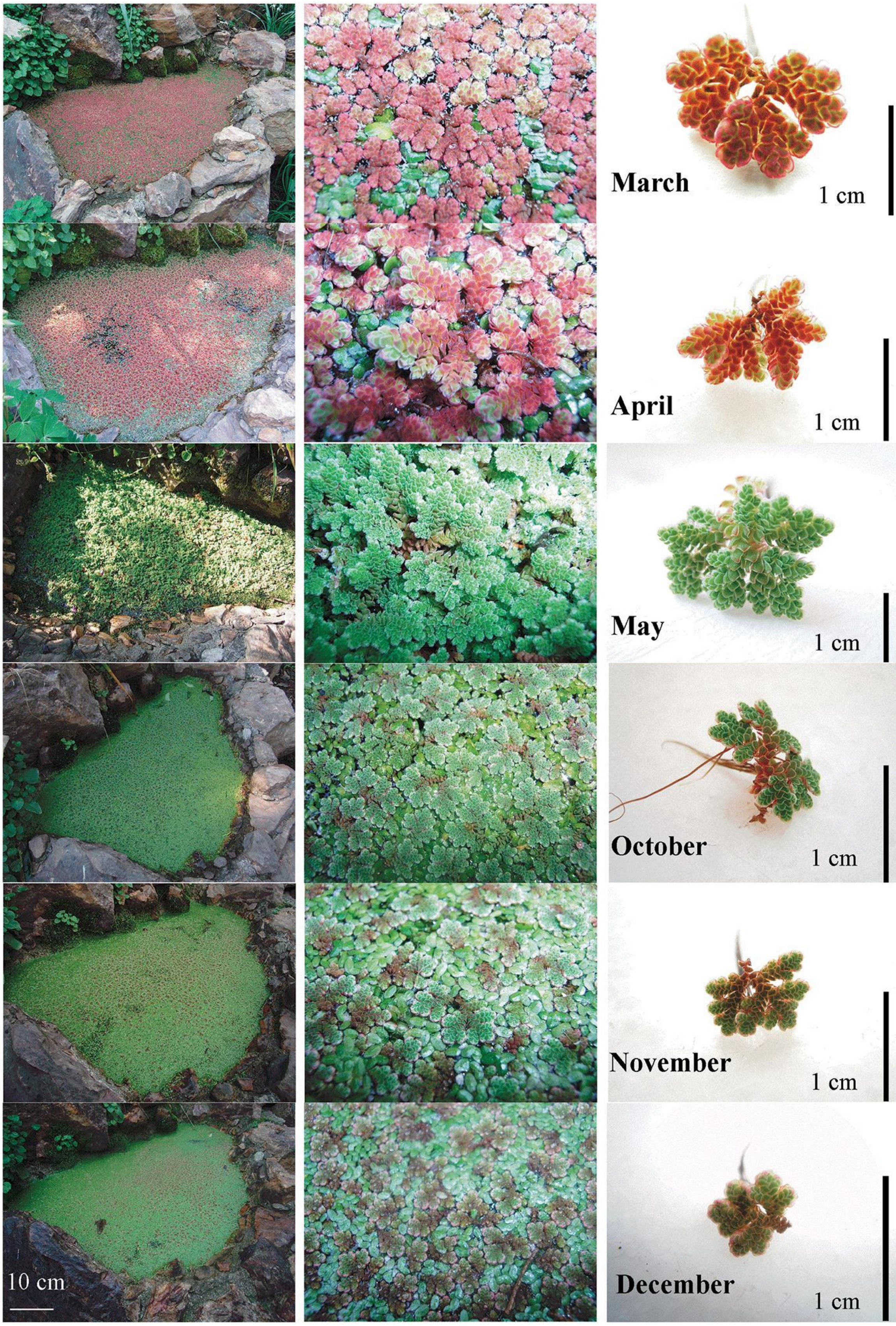

Figure 1.5: Effect of changing seasons and climate on the shape and color of Azolla.

1.2.6 Wind and Waves

Other factors that affect aquatic plant production include wind and waves. Particularly in the case of Azolla, which is a floating fern and it can be disturbed easily by wind and waves. In shallow lakes, wind induced winds can have direct and indirect effects on plant growth, for example direct effects such as mechanical damage, and indirect effects such as increased silt (Vermaat, Santamaria et al. 2000, Santamaría 2002).

Since Azolla is a small, freely floating plant, stirring the water can fragment fronds and damage delicate roots. So wind and waves don't help it grow. This fragmentation can have a negative effect on N_2 fixation ability. Therefore, wind and turbulent waters can break apart and kill Azolla. Additionally, big waves generated with hurricanes are very harmful to its growth. We can protect Azolla from winds and waves with dams, debris, wooden buoys and large buoys like Phragmites and Typha.

1.2.7 Salinity

Various studies on different Azolla species showed the negative effect of salinity on the growth of these species. There are not yet in-depth studies on the variability in salt tolerance of different types Azolla. In general, however, this genus is very sensitive to sodium chloride (Rai and Rai 2000, Fernández-Zamudio, García-Murillo et al. 2010).

Rajrathinam and Padhya (1989) reported that *A. filiculoides* can tolerate a salinity of up to 20 mM NaCl but *A. pinnata* bears almost twice as much as it can bear 40 mM NaCl in the medium. Inhibition of Azolla growth (fresh and dry weight) occurred when NaCl concentration was increased to 40 and 50 mM. Azolla was killed within two weeks in the presence of 50 mM NaCl .

Masood, Shah et al. (2006) reported that salinity inhibits the growth of *A. pinnata* and *A. filiculoides*, which leads to a significant decrease in dry weight. Therefore, with increasing NaCl concentration, the growth of *A. pinnata* and *A. filiculoides* gradually decreased. *A. filiculoides* was more sensitive to higher salt concentration than *A. pinnata*. Salt concentrations above 10 mM NaCl inhibited the growth of *A. filiculoides*, but *A. pinnata* stopped growth at 40 mm NaCl.

The presence of certain nutrients such as nitrates in salty habitats plays a major role in transmitting salt tolerance to Azolla (Mishra and S. Singh 2006). Fernandez et al. (2010) studied the germination characteristics and germination success of spores in *A. filiculoides*. The germination rate showed a significant decrease with increased salinity.

Arora and Singh (2003) conducted a study on six different Azolla species (*filiculoides, mexicana, microphylla, pinnata, rubra* and *caroliniana*) in New Delhi. Based on observations, *A. microphylla* showed higher salt tolerance than other species. In general, salinity greatly reduced biomass production in all six species.

Khosravi, Taghi Ganji et al. (2005) studied the toxic effect of heavy metals (Pb, Cd, Ni, Zn) on the growth of *A. filiculoides* in the Anzali wetlands (Iran). It has been mentioned that the removal of heavy metals from the water by Azolla can be reduced if the salt concentration in the water (in terms of NaCl) is increased. In other words, there is a decrease in the absorption of heavy elements due to the high level of salinity that can prevent Azolla Growth (Arora and Singh 2003, Fernández-Zamudio, García-Murillo et al. 2010). Sadeghi, Zarkami et al. (2012b) found that salinity could play a role An intermediate role in the growth of *A. filiculoides* in the Anzali wetlands. The nitrogen content of Azolla fronds was gradually decreased by increasing the salt concentrations up to 40 and 50 mm NaCl but was toxic to Azolla spp at 85 mm NaCl (Tantawy and Herzalla 2003).

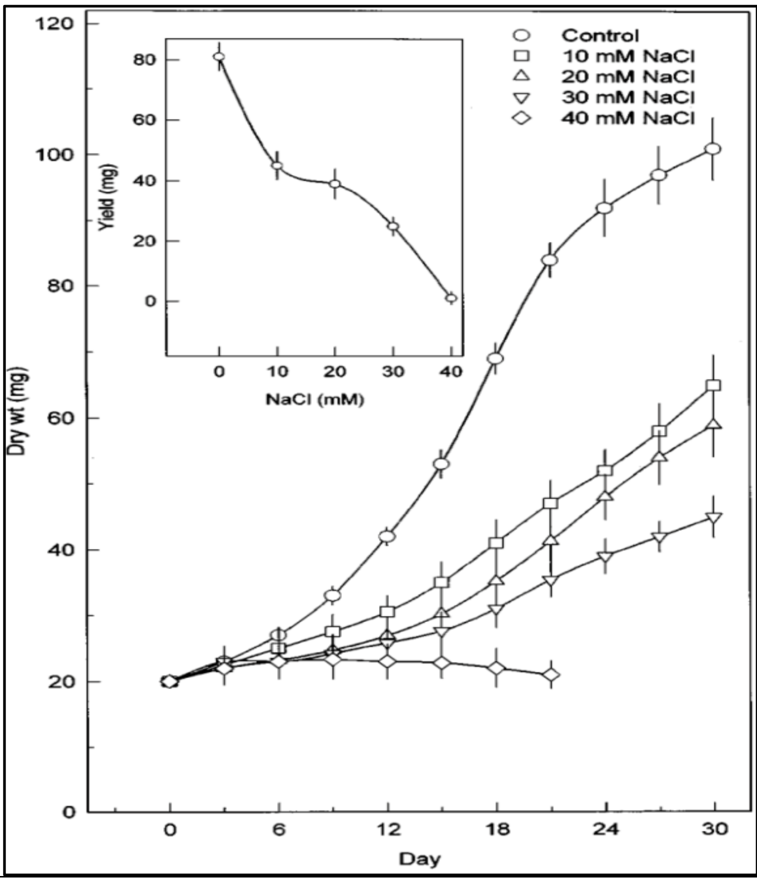

Figure 1.6 : Growth of *Azolla pinnata* at various salinity levels (Rai and Rai 2004).

1.2.8 pH

Azolla response to pH depends on many factors such as temperature, nutrients (nitrogen and phosphorous), light, and the presence of iron and soil (Wagner 1997). The optimum pH for Azolla growth typically ranges from 4.5 to 7.5, as Azolla prefers a medium close to neutral or to some extent acidic conditions (Cary and Weerts 1992). Nevertheless, *A. filiculoides* and *A. pinnata* can grow well at pH ranges between 5 and 7, *A. filiculoides* grow well only in the range between 5 and 7, while *A. pinnata* can grow relatively well in the pH range between 5 and 8 (Cary and Weerts 1992).

Watanabe, Espinas et al. (1977) found that Azolla growth decreased by increasing the pH level at low iron concentration. Whereas, in the iron deficient environment the growth and nitrogen accumulation were lowest at pH 7.5. Meanwhile, the pH is higher than 6.5, and in the same iron-deficient environment the fronds turned yellow. The optimum pH at high light intensities is 9-10. While the optimum pH for low light intensity is 5-6 (Tuan and Thuyet 1979, Lumpkin and Plucknett 1982).

Lumpkin and Plucknett (1982) reported that highly alkaline soils and groundwater would lead to reduced phosphate availability due to calcium and magnesium precipitation. Availability of most of the micronutrients for Azolla it increases as the pH decreases. Also, increasing the solubility of aluminum, iron, and manganese with increased acidity may cause toxicity and may interfere with the absorption of calcium, magnesium and other essential cations. Unlike most other micronutrients, molybdenum deficiency may occur from decreasing in pH.

El-Haddad, Mahmoud et al. (1988) explained that the growth of *A. pinnata* and *A. filiculoides* was not suitable at pH 3 as they failed to grow at this pH. But *A. pinnata* was more resistant to pH changes than *A. filiculoides*. They found that at a pH of 8 the maximum dry matter content was obtained for both Azolla species.

In greenhouse experiments, Wagner (1997) found that *A. pinnata* and *A. filiculoides* showed maximum growth at pH between 5-7 at water temperature of 25 °C. On the other hand, *A. pinnata* showed greater tolerance to a wide pH range compared to *A. filiculoides*, the latter growing more weakly at pH 4 values. And 8. Meanwhile. Dawar and Singh (2001) reported that when neutral pH is applied, N fixation decreased.

Serag, El-Hakeem et al. (2000) show that the optimum growth for cultures is at pH between 4.5 and 7, but even at pH values ranging from 3.5 to 10. Moses (1994) showed that under Egyptian environmental conditions Azolla grows well with a pH ranging from 7.1 to 9.0. In conclusion, the optimum growth in Azolla depends on other environmental conditions and not just on the pH.

1.3 Mineral Nutrition

Like most plants, Azolla is sensitive to changing deficiencies in the supply of plant nutrients. For optimal growth, ferns require all the macronutrients and micronutrients required for normal plant growth (Moore 1969) .

Yatazawa, Tomomatsu et al. (1980) found that the threshold levels of P, K, Mg, and Ca^{+2} required in the medium for Azolla growth were about 0.03, 0.4, 0.4, and 0.5

mmol^{-1}, respectively. Where complete nitrogen activity requires 0.03, 0.6, 0.5 and mmol^{-1} respectively. On the other hand, the breakdown levels of iron, manganese, molybdenum and micronutrients for the growth of Azolla were 50, 20, 0.3 and 30 g, respectively. Meanwhile, macronutrients such as N, P, K, Ca^{+2} and magnesium are especially important and have noticeable effects on fern growth, especially if it is present in very high or very low concentrations. Usually, the nutrients should be available in the water, although in very shallow waters, the Azolla plant may extract some nutrients from the soil. Furthermore, adequate plant nutrient levels must be maintained throughout the growth period (Tung and Shen 1985)

1.3.1 Macronutrients

1.3.1.1 Nitrogen

Nitrogen availability and to some extent, dissolved oxygen are not a determining factor in Azolla growth. This is unlike other minerals, but the growth of algae-free plants depends On common nitrogen. According to the study of Sadeghi, Zarkami et al. (2012a) increased nitrate concentration may lead to reduced Azolla coverage in Anzali wetlands.

The reason is that Azolla can have free access to atmospheric nitrogen (N_2) to satisfy all of its requirements through its symbiosis with Anabaena, while plants devoid of algae are deprived of such opportunities (Costa, Santos et al. 2009). Azolla's upper lobes have free contact with air, which is important for nitrogen fixation. High ammonium concentrations in wastewater had no effect on nitrogen fixation dynamics, based on a studies of Costa, Santos et al. (2009) surveyed nutrient flows along the roots of 4 wetland plants (Azolla spp., *Vallisneria natans*, *Bacopa monnieri* and *Ludwigia repens*) in order to see the dynamics of nutrient removal from nutritious water systems. And when different ions (such as NH^{+4}, NO^{-3}) were tested, Azolla show a preference for NO^{-3} uptake.

Growth and nitrogen fixation under field conditions were estimated at approximately 3.1 to 4.6 kg N/ ha/ day in *A. pinnata*. During day 15, a seven to eight-fold increase in biomass was observed doubling time 4.8 days (Kulasooriya, Hirimburegama et al. 1982). Most reports state that N_2 fixation rates range from 0.4 to 3.6 kg N/ ha/day depending on the absence/presence of nitrogen fertilizers (Watanabe 1982, Cary and

Weerts 1992). Based on the studies of (Cary and Weerts 1992, Biswas, Parveen et al. 2005, Costa, Santos et al. 2009) nitrogen accumulation is estimated to be 5.0-9.0 mg N per gram dry weight per day when field conditions are ideal. This can lead to a dry matter gain of 0.135-0.290 g per gram of Azolla dry weight per day. This value corresponds to a doubling time of 2.5-5.5 days.

1.3.1.2 Phosphorus

Phosphorous is one of the most important nutrients that often limit Azolla growth if it is less than the required concentration. Phosphorous deficiency is indicated in younger and less active plants and may cause plants to turn pink to deep red, brittle, and develop roots for too long. It has been found that if the phosphorus concentration is less than 0.6 ppm in the nutrient solution, this reduces the growth rate, nitrogen fixation and chlorophyll content in *A. pinnata* (Cohn and Renlund 1953, Subudhi and Watanabe 1979). Like many other aquatic organisms with photosynthesis, phosphorous (in the form of phosphate) is one of the main nutrients that limit the growth of Azolla. Phosphorous is An important nutrient for the successful and rapid growth of this species (EL Katony, MS et al. 1996)

The effect of this very important nutrient on the overgrowth of Azolla in the Anzali wetlands has been demonstrated in laboratory experiments (Sadeghi, Zarkami et al. 2012a, Sadeghi, Zarkami et al. 2012b). Janes (1998) has found that increased phosphorous supply and/or plant density led to increased ovulation. Azolla will be able to grow without the need to provide co-nitrogen such as NH_4NO_3, If there is enough phosphorous in aquatic organisms environment (Costa, Santos et al. 1999).

There have been various reports about the phosphorous levels required to continue Azolla growth. In laboratory experiments, the concentration was approximately 0.06 ppm (2 μM) and it was sufficient to sustain Azolla growth. From a field surveys a range of 0.3 to 1 ppm (10 to 33 μM) has been suggested. The optimum growth of different Azolla species responds to different concentrations of phosphorous (Kushari and Watanabe 1992).

Ramirez (1984) concluded that *A. pinnata* can thrive without phosphorus use in soil according to Olsen phosphorus indicator, it has been found it is greater than 30 mg/kg and phosphorus absorption energy less than 1500 mg P_2O_5/100 g soil. They have suggested that factors other than phosphate may influence the growth of Azolla. At the same time, they have noticed a large variation in Azolla growth between different soils with similar available phosphate contents. Ito and Watanabe (1985) reported that 25 ppm of phosphorus in soil is optimal for Azolla growth. The phosphorous content of Azolla grown in pots of different types of soil and in the field showed that the limit value for phosphate deficiency was 0.4% P in Azolla (on a dry weight basis), 20 ppm phosphorus in soil, and 0.15 ppm phosphorus in flooded fields .

1.3.2 Micronutrients

The importance of certain micronutrients for the successful and rapid growth of Azolla has long been recognized, elements such as iron and trace elements such as Molybdenum, particularly in relationship with nitrogen fixation (Biswas, Parveen et al. 2005). Nitrogen fixation by Azolla-Anabaena requires cobalt and molybdenum (Zahran, Abo–Ellil et al. 2007).

Singh, Mishra et al. (2010) studied the effect of some micronutrients (Mo, Mn^{+2}, Zn^{+2}, Cu^{+2} and Fe^{+2}) on the cellular and extra-cellular activities of two Azolla species (*A. microphylla and A. filiculoides*) exposed to brine water (20 mM NaCl). The micronutrients showed a significant improvement in the specific activity at low concentrations (0-0.01 mmol), while higher concentrations (e.g. 10 mmol) played an inhibitory role. Sadeghi et al (2012b) reported an effect on the growth of *A. filiculoides* in the Anzali wetland, to a moderate degree.

Wagner (1997) found after examining that the marginal levels of micronutrients such as Fe, Mn, Mo and B for Azolla growth were 50, 20, 0.3 and 30 μg/L, respectively. The availability of iron that can be accessed by Azolla itself is affected by pH values. Iron precipitates as hydroxide so that it becomes less available to Azolla when the pH values is high, and when Azolla cannot reach iron, it turns yellow. Jain, Gujral et al. (1992) studied the toxic effect of some elements on Azolla, their study showed that copper, cobalt, lead and zinc have a toxic effect on the growth of Azolla. While iron and manganese do not have any toxic effect on the anaerobic fermentation of Azolla. Micronutrients such as molybdenum, iron and cobalt are required for nitrogen fixation (Peters 1991, Jain, Gujral et al. 1992).

1.3.2.1 Iron

Because iron is a fundamental component of nitrogenase, it is considered a common determinant (Stanier 1974). Watanabe and Espinas (1976) reported that 1 ppm iron was sufficient for the rapid growth of Azolla. The critical iron concentration for the growth of Azolla was 20-50 μg in 1. Whereas for pH 4 the ferric ion was so readily available that a higher concentration of calcium was required to balance the increased iron absorption. Otherwise, the fronds suffered from iron toxicity (Lumpkin and Plucknett 1980). Likewise, in case of iron deficiency, plants turn yellow due to the depletion of chlorophyll. The roots become thin and white (Malavolta, Acorsi et al. 1981). Iron availability decreases due to neutral to alkaline conditions (Lumpkin and Plucknett 1980, Watanabe 1982). *A. filiculoids* require iron for growth and N_2 fixation, that was increased at a level of up to 5 mg/1 as Fe-EDTA. At high pH values the problems with iron absorption are worsened, as ferric ions precipitate and become unavailable for plant uptake (Singh, Misra et al. 1984, Wagner 1997)

1.3.2.2 Cobalt

Cobalt is required for nitrogen fixation and symbiotic growth of Anabaena-Azolla in the host plant. Azolla growth, chlorophyll content and nitrogen fixation were increased by adding cobalt in the absence of compound nitrogen (Johnson, Mayeux et al. 1966, Lumpkin 1987).

1.4 Uses and benefits of the Azolla plant

Azolla has gained prominence in recent years. The concept of using aquatic plants for various purposes is receiving special attention nowadays. Because of their high reproduction rate, breeding habitat, being an excellent source of protein for monogastric animals, their high production rate of biomass, increased demand for it as an organic food and for their wide range of uses in food industry either for animals and humans (Raja, Rathaur et al. 2012).

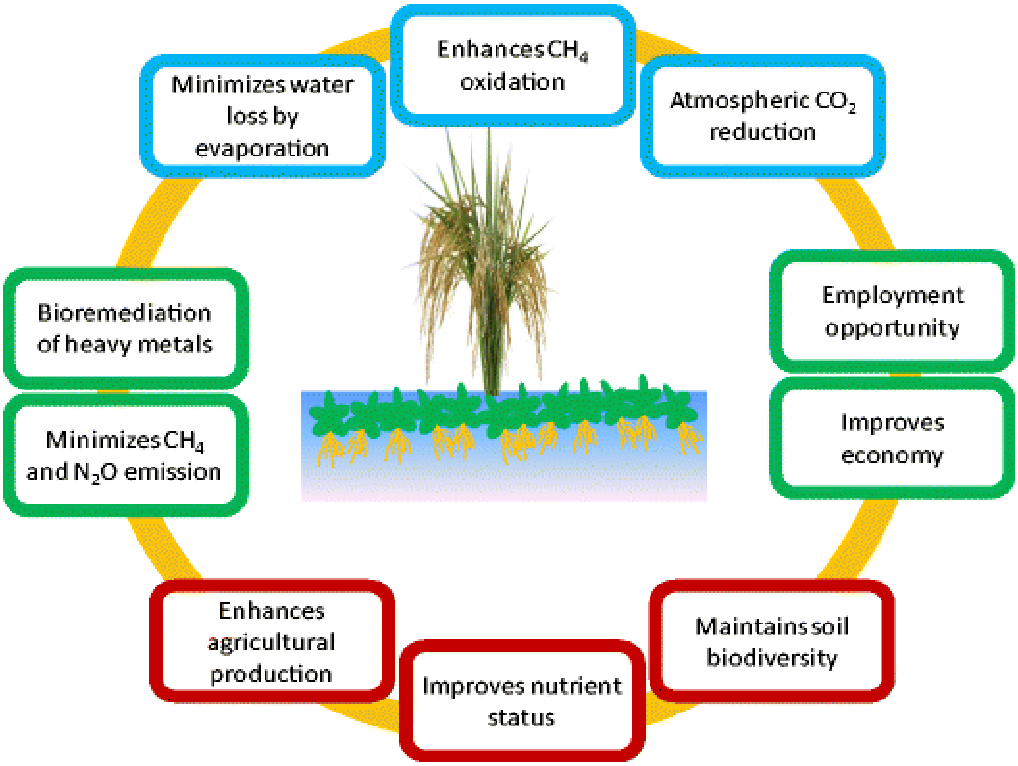

Figure 1.7 : Some Agricultural And Environmental Benefits And Uses Of Azolla

1.4.1 The use of Azolla as a biofertilizers

The use of Azolla as a biofertilizer play an important role in maintaining and improving the state of the global environment. Through biotechnology interventions, the potential of the pteridophyte could be exploited more efficiently in the future (Amit, Amit et al. 2016)

Azolla can be used as a biofertilizer, as one hectare produces 300 tons of green biofertilizer annually in a normal subtropical climate, which is equivalent to 800 kg of nitrogen (1800 kg of urea). The rapid decomposition of the soil and the effective availability of nitrogen in the rice are an important factor in the use of Azolla as a biofertilizer for the rice crop. The fast multiplication rate and fast decomposition ability of Azolla has become a critical factor for use as a green biofertilizer with biofertilizer in paddy field. The benefits of applying Azolla in paddy field are as follows, the base application of green manure at 10-12 t/ha increases soil nitrogen by 50-60 kg/ha and reduces 30 to 35 kg fertilizer nitrogen requirement for rice yield. Green Azolla release double the rice crop cultivation rate of 500 kg/ha. It also contributes to enriching soil nitrogen by 50 kg/ha and also plays an important role in reducing nitrogen requirements by 20-30 kg/ha. Finally, the use of Azolla increases the rice yield by 20-30% (Widiastuti 2017). Safriyani et al. (2020) results showed better growth in stem height and collar diameter and good performance with *Azolla filiculoides* biofertilizer especially in the Sunny cultivar. It is concluded that the area of leaves is increased.

Many researches have been carried out on Azolla during the past and recent decades as it has been proven that it has the ability to fix nitrogen in the atmosphere as well as to produce biomass at a very high rate. Azolla is commonly used as a biofertilizer as well as green fertilizer in paddy fields (Amit, Amit et al. 2016). And because Azolla plays an important role in fixing nitrogen from the atmosphere, planting it in the paddy field is one of the strategies to improve the efficiency of nitrogen fertilizers and increase the vegetative and generative growth of the plant (Raja, Rathaur et al. 2012). it is concluded that Azolla could be used as a sustainable biofertilizer for vegetable production in acidic tropical soils in dry lands, in order to increase production and soil fertility. Moreover, it was also found that Azolla and manure could be used to enhance yield and nutrient concentrations in radish and spinach crops, in addition to improving soil fertility in alluvial and peat soils, enhancing microbial communities in the soil and reducing abiotic microbial stress (Roy, Pakhira et al. 2016).

In summary, results highlight the importance of using nitrogen fixing organisms, such as Azolla, which can effectively help improve more sustainable agriculture in developing countries, without the risk of problems associated with the adverse effects of long-term soil fertility, soil productivity and environmental issues from the use of chemical fertilizers (Widiastuti 2017, Safriyani, Hasmeda et al. 2020).

The general characteristics that make Azolla suitable as a biofertilizer in rice can be outlined as the follows:

1- Azolla can stabilize nitrogen at a great rate.

2- Azolla has a fast growth rate.

3- Because Azolla floats on the surface of the water, it cannot be supplemented with rice for light and space.

4- In most climates, Azolla can grow better under partial shade

5- Of the vegetation that the rice canopy can easily provide in their early and middle stages of growth.

6- Azolla begins to die and decompose, thus releasing nutrients into the medium. When the rice is near maturity, due to the intensity of low lighting under the canopy and depletion of nutrients.

7- Azolla has a fast degradation rate, so the reformed nitrogen, phosphorous and other nutrients that you may have noticed from the water, and perhaps in competition

8- with rice are rapidly released back into the medium and made available for the absorption of the rice as the grain grows .

9- Azolla has a greater ability than rice to accumulate potassium in its tissues in a low-potassium environment. Hence, after decomposition, it makes this nutrient available for rice.

10- The thick Azolla mat in the rice fields contributes to weed elimination (Hechler and Dawson 1995, El-Shahat 1997, Carrapico, Teixeira et al. 2000, Mwingira, Mayala et al. 2009).

Figure 1.8: The use of Azolla as an organic fertilizer in rice fields.

1.4.2 Azolla as Green Manure

Azolla can also be used as a green fertilizer in the cultivation of hydroponic bamboo, taro, arrowhead, rice and wheat. Incubation of Azolla in flooded soil as green manure resulted in rapid mineralization with the release of 60-80% nitrogen within two weeks. Some studies recorded the highest wheat yield with application of 20 tons of Azolla and 60 kg of nitrogen (Raja, Rathaur et al. 2012, Amit, Amit et al. 2016)

1.4.3 Azolla as a Component of Space Diet

Recent research by Katayama et al. In cooperation with the Space Agriculture Task Force, 2008, the Azolla plant can be considered an important component of the space food system while inhabiting the surface of Mars. It meets the basic and essential nutritional requirements of a human being on Mars (Roy, Pakhira et al. 2016)

In July 1980, Vietnamese astronaut Phạm Tuân took samples of Azolla to the Soviet space station Salyut-6 in order to visualize and determine its potential future uses in space travel and planetary colonization. Both of these habitats require closed-loop life support systems (CLLS), also known as Controlled Environmental Life Support Systems (CELSS). Due to the limited space available in these habitats, the Azolla is ideally suited to CELSS. Where the Azolla plant can live in a water depth of 2 to 3 centimeters (about one inch), so it can be grown in multi-layered frames that require less space than other plants and thus provide more space. This gives it an additional advantage over some of the other suggested plants such as sweet potato, which has also been suggested for use on space stations because Azolla is considered edible. However, plants like sweet potatoes require a relatively large growth area and take several months to ripen, as do rice and wheat, which are other candidates for food in space. This contrasts with Azolla's ability to double its biomass in just two to three days (Liu, Min et al. 2008).

A Chinese team led by Xiaofeng Liu has conducted numerous and varied experiments to determine the ability of Azolla to provide needed and sufficient oxygen within a "Azolla-human" ecosystem of CELSS. Their results showed that, in CELSS, 16 m^2 Azolla plants were able to provide needed and sufficient oxygen for two adults to use (Liu, Min et al. 2008). A number of western scientists such as Francisco Carrapiço at the University of Lisbon, have published his research on Azolla plant, in which he recommends the use of Azolla in space and the colonization of plants.

Based on the results of these studies, it is not difficult to imagine environments enclosed in space or on the surface of other planets that contain layers of shallow trays filled with green floating plants (Azolla) that provide the human population with oxygen and food (Carrapico, Teixeira et al. 2000).

1.4.4 Azolla As A Human Food

It is well-known about the Azolla plant that it is used as fodder for livestock, especially in India and the Middle East countries, or as fertilizer in rice field, but the possibility of using it as food for people is less known. This is changing now and some motivations has come from studies in food systems that can be used for space stations, space travel, and dwellings on the Moon and Mars. Azolla's protein content is close to that of soybeans. It is a rich source of minerals (10 - 15% of dry weight), essential amino acids (7-10% of dry weight), vitamins and carotenoids. 20 - 30% of the dry weight of Azolla is protein, which is a lot for a vegetables, and the protein quality in Azolla is good. Li shizhen published a book in China in the 16th century describing the medicinal properties of Azolla, which encouraged people to consume it as food (Raja, Rathaur et al. 2012, Amit, Amit et al. 2016, Roy, Pakhira et al. 2016).

Azolla recipes

Erik Sjödin includes many recipes that he developed that use Azolla as a main ingredient for their preparation. Examples of these delicious recipes are Azolla Soup, Azolla Burger, Azolla Balls, Azolla Pies, Hard take Azolla, and Azolla Bread (Sjödin 2012).

Figure 1.9: Erik Sjödin Azolla recipes

1.4.5 Azolla as Mosquito Repellent

Azolla is known by several names, including "mosquito fern" due to its ability to reduce mosquito reproduction numbers by more than 95%. It does this by covering the surface of bodies of fresh water that are slow, static, or stagnant, which makes adult mosquitoes unable to lay eggs and also reduces the appearance and development of mosquito larvae. This reduces the risk of mosquito-borne epidemics and potential epidemics such as malaria, chikungunya virus, eastern equine encephalitis, Saint Louis encephalitis, lacrosse encephalitis, western equine encephalitis, canine heartworms, dengue fever, yellow fever, West Nile virus and most recently, Zika virus (Okech, Mwobobia et al. 2008, Mwingira, Mayala et al. 2009, Raja, Rathaur et al. 2012, Amit, Amit et al. 2016, Roy, Pakhira et al. 2016).

The breeding of mosquitoes that transmit malaria in the Indian city of Ghaziabad, near New Delhi, was almost completely suppressed in ponds, wells and ponds covered with Azolla (Ansari and Sharma 1991). Likewise, immature mosquito numbers have decreased dramatically in southern India, by means of Azolla mats covering the waters in rice fields (Rajendran and Reuben 1988).

In Africa, Mwingira, Mayala et al. (2009) reported that Azolla reduced egg laying and adult mosquito breeding in Tanzania, due to reduced larval productivity in sites with high Azolla cover (more than 80%) of the water surface. And in Kenya, Okech et al. (2008) also showed that Azolla is able to form a dense canopy in paddy fields. He said his famous phrase: "smothering mosquito larvae while nourishing rice growth".
Reducing mosquito numbers can also be promoted by adding freshwater fish, such as carp or tilapia, which all feed on mosquito larvae and Azolla plants, thus contributing effectively to providing a local and renewable source of protein-rich food. Also, the presence of Azolla on the surface of water bodies does not have a detrimental effect on the water quality. Azolla actually has a beneficial effect by improving water quality.

1.4.6 Azolla as Nutritional Supplement for Livestock

Azolla can be used as a major and important component in the diet of a wide range of animals, such as birds of all kinds, pigeons, broiler chickens, laying hens, ducks and geese, in addition to sheep, goats, cows, buffaloes, pigs, and many others (Amit, Amit et al. 2016, Kumar and Chander 2017, Kumar, Reager et al. 2020, Kumar, Reager et al. 2020)

1.4.6.1 Use as Cattle Feed Supplement

Kumar, Reager et al. (2020) has conducted a study to find out the effect of feeding using Azolla on milk production for lactating cows under field conditions. The results showed an increasing trend and a marked increase in the percentage of milk production, as the rate increased to 9.90 liters/day from 8.30 liters/day after 90 days of feeding Azolla daily. The data and results of the study revealed that there is an increase of 19.51 percent in milk production for cows compared to cows fed using traditional fodder. Singh et al (2017) also reported increased milk production in buffalo, and fat percentage has increased by 1.96% in cows.

1.4.6.2 Broiler chickens

Adding Azolla to conventional poultry feed significantly saves production costs. Azolla is an alternative, cheap and abundant source of plant protein. In addition, the introduction of Azolla into the traditional diet improves the rate of nutritional conversion, energy efficiency, and performance with no harmful effects on livestock, poultry and humans (Lejeune, Cagauan et al. 1999, Alalade and Iyayi 2006, Namra, Hataba et al. 2010). Most of the data show an improvement in the production and breeding of livestock and poultry fed an Azolla diet, although not all studies are consistent (Namra, Hataba et al. 2010).

Incorporating Azolla as a feed ingredient of up to 5% to replace sesame flour in 2 to 6 week old rations of poultry resulted in a significant improvement in growth rate, FCR, and also in energy efficiency without any adverse effects on palatability and mortality with promising economic returns (Basak, Pramanik et al. 2002, Parthasarathy, Kadirvel et al. 2002). Fresh Azolla has been suggested as an alternative to commercial feed in 20% chicken feed because it can increase body weight (Sherief and James 1994). 20% when Azollain was increased by up to 15% in the diet (Querubin 1986). The study of Dhumal, Siddiqui et al. (2009) showed that when there was an increase in Azolla levels by up to 30%, there was a significant increase in feed intake. Ali and Leeson (1995) indicated a benefit from Azolla supplementation at a low level of inclusion. Likewise, Ara, Adil et al. (2015) found a linear decrease in forage intake with increasing levels of Azolla in broiler poultry feed.

It has been found that Azolla pinnata can be safely included in rations of developing beans without health problems of up to 15%, but the best performance was when the inclusion level is of 10% in the chickens' diet. Incorporation of Azolla at a level of 7.5% with the concentrated feed resulted in a body weight gain of 2.6% (1.99 kg) Compared to the control diet (1.93 kg) (Alalade, Iyayi et al. 2007). In addition, the feed consumed was less on broiler feed by 7.5% Azolla (Prabina and Kumar 2010). Seth, Pradhan et al. (2013) also discovered that there was an increase in the weight of the Vanraga chickens feeding 5% or 10% more Azolla than the control.

Figure 1.10: Chicken feeding on Azolla

Saikia, Sapcota et al. (2014) showed that the increase in body weight was the highest possible in the group supplemented with 5% Azolla while the lowest increase was in the group fed with a level of 15% and explained that increasing the fiber content in high concentrations of Azolla reduces the growth rate due to it adversely affecting On the appetite. They also concluded that Azolla could be added to broiler poultry feed at 10% without causing any negative effect on performance. A significant improvement in body weight gain as well as FCR after Azolla was included was detected at a level of 5% or 10% in the forage (Acharya, Mohanty et al. 2015) and up to a level of 7.5% (Kumar, Dhuria et al. 2018).

It has been revealed that Azolla pollination at 5% or 7% levels is suitable for safe and profitable natural production of broiler chickens due to lower feed conversion, mortality and production costs at the same time, in addition to improving net profit (Islam and Nishibori 2017). More recently,Samad, Idris et al. (2020) has suggested that the addition of Azolla can enhance the growth performance traits without negative effects on the digestibility of the nutrients of broiler chickens by adding it at a concentration of 15%.

1.4.6.3 Layer chickens

The dyeing ability of Azolla was inferred and demonstrated after observing the Azolla-fed group by the higher Roche fan color tone, and this could be attributed to the Azolla's ability to stain yolk due to its richness in-carotene dye. Bastian (1987) noted a similar effect on the color of the yolk due to the inclusion of Azolla in the toppings. Ali and Leeson (1995) found that adding servings of Azolla powder to chicken feed significantly improved the carotene status of the chickens and increased egg production. Moreover, Khatun, Ali et al. (1999) investigated the possibility of substituting sesame oil in layered rations by Azolla 200g/kg and the result was his finding of good egg mass production and FCR. A comparable better egg production performance was discovered by Kannaiyan and Kumar (2005) in terms of a higher egg production rate after including rations of Azolla at a level of 100 g/bird/day.

Figure 1.11: Feeding laying hens on Azolla

Lakshmanan, Kumar et al. (2017) reported that Azolla supplementation in the stratified diets resulted in a higher rate of egg production as well as improved nutritional value and the maintenance of concentrated feeds. The positive effect of using rations of Azolla in fodder on eggshell resistance was indicated due to the high calcium content in Azolla. It has been found that eggshells are composed of minerals especially calcium carbonate which are deposited in the organic matrix (Austic and Nesheim 1990). It was observed and reported that when inoculating rations of Azolla into rations of laying poultry feed at levels up to 20% had no effect on levels of alanine aminotransferase and aspartate aminotransferase, indicating that there was no toxic effect of Azolla cristata supplementation (Ara, Adil et al. 2018) .

It can be concluded that Azolla species can be used as a source of protein and other essential nutrient elements for poultry species. Further research is required to determine the effects of using Azolla as a feed supplement in poultry ration.

1.4.6.4 Ducks

Several studies also showed the effect of using Azolla as a nutritional supplement on duck rations. Azolla can be added as an effective and unconventional forage source of 10% nutrient to the basic diet of white Pekingese ducks (Acharya, Mohanty et al. 2015).

Figure 1.12: Breeding ducks in Azolla ponds

In Escobin Jr (1987) study, the author did not notice any differences in production efficacy between different groups after performing partial substitution of rations of growing muscovy ducks with Azolla at different levels of 20, 30 and 40% . In addition, a 15% replacement of soybean powder with Azolla increased the daily gains for ducks (Becerra, Preston et al. 1995). However, a decrease in the growth rate was observed at levels 20, 45, or 60% of Azolla. Similar results have been reported on duck production performance (Lawas, Roxas et al. 1998, Sujatha, Kundu et al. 2013). In the same line, Lawas, Roxas et al. (1998) observed that feeding mallard ducks with Azolla at levels of 75 or 250 g/ bird/day lowers the rate of feed conversion .

In an experiment with ducks, Becerra, Preston et al. (1995) demonstrated that by adding 15 to 60% Azolla to the diet providing 15.2-30.3% of total protein, the rate of nutritional conversion could decrease with the increase in Azolla consumption . It was found that when using fresh Azolla pinnata supplements at 200g/ duck/day, they replaced 20% of the standard duck layer feed. It will improve the egg production, egg weight, feed conversation ratio, performance efficiency index and shape index with enrichment of yolk color (Acharya, Mohanty et al. 2015).

1.4.6.5 Quails

Shamna, Peethambaran et al. (2013) Feeding quails on Azolla could enhance growth and FCR at a substitution level of 5% of the base ration, and compared to feeding on the basic diet alone was more economical. Varadharajan, Gnanasekar et al. (2019) concluded that a quail diet that includes an Azolla meal of up to 6% does not affect feed consumption. Rathod, Tyagi et al. (2013) reported some insignificant effect of Azolla supplementation on the rate of Japanese quail feed consumption .

1.4.7 Azolla: Beneficial Effects on Crops

Azolla is beneficial for wheat when used in a rotating rice wheat crop system. Researchers found that applying Azolla with Sispania had a beneficial residual effect on subsequent wheat crops, increasing the grain yield 56-69% more than the control. In other studies, researchers found that the incorporation of 6, 12, 18 and 24 tha-1 of fresh Azolla into the soil significantly and significantly increased the ability to retain water, organic carbon, ammonium, nitrogen, phosphorus, potassium and available calcium. Magnesium, while in turn reducing the pH and bulk density, this combination significantly increased the yield of mung beans (Raja, Rathaur et al. 2012, Amit, Amit et al. 2016) .

Figure 1.13: Azolla beneficial Effects on Crops

1.4.8 Azolla: Beneficial Effects on Physio-chemical Properties of Soil

Azolla is used to increase soil fertility. Several researchers have found that applying Azolla improves soil fertility by increasing the total nitrogen, organic carbon and phosphorous available in the soil, and other researchers have found that Azolla plays an important role in improving soil structure (Raja, Rathaur et al. 2012, Amit, Amit et al. 2016).

1.4.8.1 Azolla in Reclamation of Saline Soils

Although Azolla is relatively sensitive to salt, planting in a salty environment for two consecutive years resulted in the salt content being reduced from 0.35 to 0.15 and the rate of desalination (71.4%) was 1.8 times faster by filtering water and 2.1 times faster than Sesbania, reduced Also, the pH of the acidic soil and the electrical conductivity also increased, and in return, the calcium content in the soil increased (Roy, Pakhira et al. 2016).

1.4.9 Azolla in Bioremediation

It was found that *A. pinnata* and Lamna removed heavy metals from iron and copper from contaminated water (Jain, Vasudevan et al. 1989). Pollutants with low concentrations can be treated by passing them through ponds and can be reused in agriculture. More recently, Arora, Saxena et al. (2006) found tolerance and vegetative accumulation of chromium by three Azolla species and also results found by Cohen-Shoel, Barkay et al. (2002) shows the biological filtration of toxic elements by Azolla biomass. Azolla exhibits remarkable ability to concentrate minerals such as copper, cadmium, chromium, nickel, lead and nutrients directly from pollutants or wastewater.

1.4.10 Suppressing the Growth of Aquatic Weeds

When Azolla forms a thick, nearly light-resistant mat, weed growth is inhibited. There are likely to be two mechanisms and methods for this suppression and prevention, and the most effective of these methods or mechanisms is to starve young weed seedlings due to blockage of sunlight. The other method or mechanism is the physical resistance to the emergence of weed seedlings resulting from a heavy and tangled Azolla mat. In some grassy rice in the fields, the benefit of Azolla weed suppression may exceed its usefulness as a source of nitrogen, and it is worth noting that the rice seedlings are not affected by the effect of Azolla weed suppression because they stand on the Azolla mat when planting (Satapathy and Singh 1985, Amit, Amit et al. 2016).

Krock, Alkamper et al. (1991) found that when mulching Azolla, the total amount of weeds decreased significantly. Also, the Azolla cover reduced the intensity of light by about 90%, which contributed to reducing the photosynthesis process in the flood waters, thus reducing the oxygen concentration in the water by more than 50%. Besides reducing the light intensity, Azolla's cover delays the light quality, and the green leaves have a filter effect that increases the relative amount of infrared. It is also noticed that the different varieties of Azolla differ in their growth rate even when grown in the same thermal environment. For example, *A. pinnata* grows faster than *A. caroliniana*, and its efficiency in photosynthesis was more effective (Satapathy and Singh 1985) .

1.4.11 The use of Azolla in the production of bioenergy

Humankind's quest to produce sustainable, renewable, and inexpensive biofuels has led to an intense search for the domestication of the next generation of bioenergy crops. Aquatic plants that can colonize wetlands quickly attract attention both because of their ability to produce large amounts of biomass and also because of their ability to grow in wastewater. The Azolla species can be considered one of the fastest growing plants, producing significant biomass when grown in polluted water and natural ecosystems. Together with its evolutionary symbionts, Anabaena azollae, it resembles terrestrial bioenergy crop groups and microalgae. Azolla biomass has a unique chemical formula that accumulates in every leaf including three main types of bioenergy molecules: Cellulose/Hemicellulose, starch and fats (Salehzadeh, Naeemi et al. 2014).

Non-polluting, high-energy fuel When Azolla-Anabaena is grown in a nitrogen-free atmosphere and/or aqueous medium containing nitrates, nitrogen is generated in symbionts, instead of nitrogen fixation, hydrogen is evolved, using water as a source. Through exposure to a micro-air environment, partial vacuum or atmosphere rich in argon or carbon dioxide, or by freezing Anabaena-Azolla cells isolated from ferns, the rate of hydrogen production can be increased (Raja, Rathaur et al. 2012, Miranda, Biswas et al. 2016).

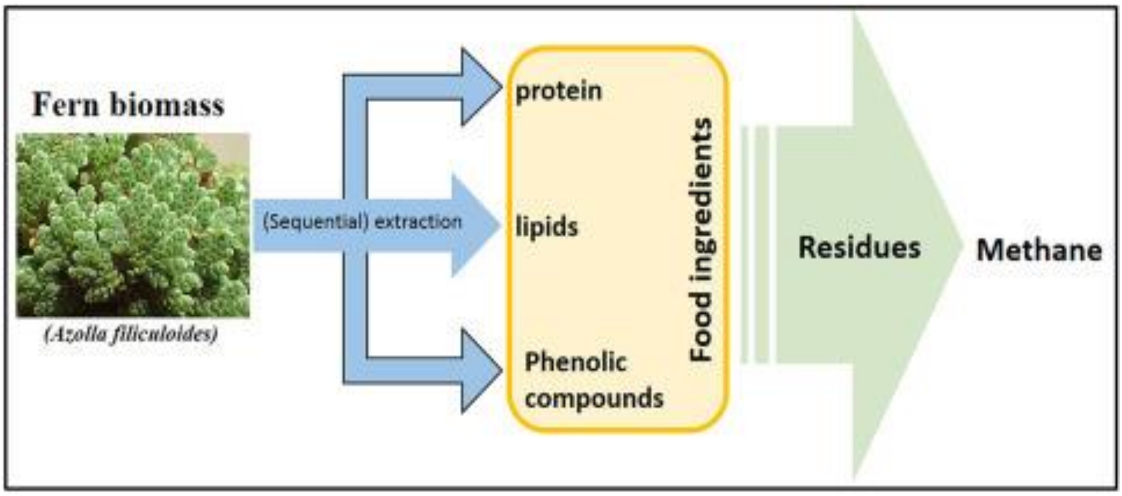

Figure 1.14: The use of Azolla in the production of bioenergy.

The results indicate that biodiesel can be produced from macroalgae and that it is possible and likely that fern, due to its ready availability and potentially low cost, could be an economical source of biodiesel (Roy, Pakhira et al. 2016).

By separate hydrolysis and fermentation with *Azolla filiculoides* as biomass, ethanol can be produced. By using pyrolysis and enzymatic hydrolysis as pretreatment methods for the production of monosaccharide's from Azolla (Miranda, Biswas et al. 2016). Finally, we can say that the ability to grow in wastewater, high productivity and unique chemical composition make Azolla the most sustainable species, attractive and universal feedstock at low cost, low energy demand, and near-zero maintenance system to produce a wide range of renewable biofuels (Salehzadeh, Naeemi et al. 2014).

1.4.12 The use of Azolla in the production of biogas

The Azolla plant can be exploited to produce methane through anaerobic fermentation of Azolla (or a mixture of Azolla and rice straw), which can be used as fuel and the remaining liquid wastes can be used as fertilizer because it contains all the nutrients already present in the plant's tissues except for a small percentage of lost nitrogen such as ammonia (Amit, Amit et al. 2016). Mixed Azolla residues with cow dung and found that the best ratio was 1: 0.4, which gave a gas yield of 1.4 times that of cow dung alone. Thus, we conclude the good effect of Azolla when it comes to biogas production (Raja, Rathaur et al. 2012)

In a study conducted, the anaerobic digestion of *Azolla pinnata* biomass grown in SIIDCUL wastewater was examined to improve biogas production. The nutrient biomass *A. pinnata* harvested after plant treatment of industrial SIIDCUL residues was subjected to anaerobic digestion for 28 days. Overall, the modeling results showed a maximum expected biogas yield (3571.14 ml), methane production (55.62%), and reduced COD (52.03%) at 35.36 ° C and 49.70% of the biomass load of *A. pinnata*, respectively. The results of this study help to increase anaerobic digestion of bio-mass *A. pinnata* and produce bioenergy in order to more sustainably meet the growing fuel requirements (Kumar, Kumar et al. 2020).

Based on experimental research, it was observed that the highest methane production was seen in crude Azolla samples with a C/N ratio of 30 pre-treated with NaOH (9%). The highest digestibility yield was obtained for the raw Azolla samples, as fumigation of Azolla is costly and time consuming, and therefore in terms of cost Azolla as a raw material is more economical and effective. The chemical pretreatment had a significant effect on the methane production. With NaOH (9%), the highest yield was obtained from the pretreated samples (Fardmanesh, Pourdarbani et al. 2020).

1.4.13 The use of Azolla in wastewater treatment

It was found that Azolla may be one of the most promising agents for COD removal and nitrogen- and phosphorous-free wastewater treatment. He conducted a study looking at the potential growth of Azolla in Secondary effluent to remove COD, phosphorous and nitrogen. The results showed that removing N and P at 100 ppm from each component in a separate medium turned out to be so 36% and 44%, respectively, while the removal of N and P decreased to 33% and 40.5%, respectively, in the mixed solution condition of these two compounds. Moreover, the results indicated a decrease in nitrogen absorption in the presence of phosphorous. Moreover, Azolla revealed a high potential to remove 98.8% COD in 28 days. Golzary, Tavakoli et al. (2018) concluded that it is possible to improve the composition of wastewater by using differences in the rates of absorption of the main nutrients of wastewater, ammonium and phosphorus by different large aquatic plants. The authors presented an attractive, environmentally friendly and cost-effective solution for efficient bio-filtration of pigs wastewater and petrochemical production from generated biomass (Muradov, Taha et al. 2014).

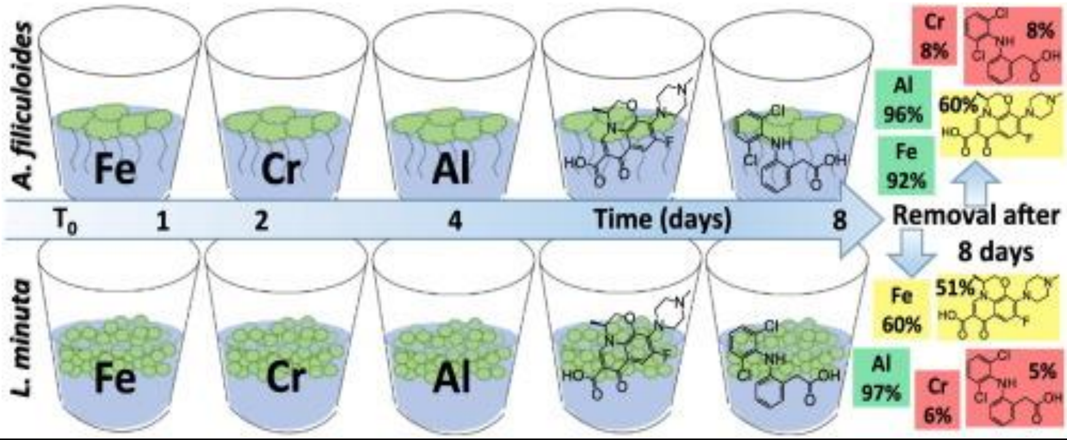

Figure 1.15: The use of Azolla in wastewater treatment.

Also *A. pinnata* can be considered effective in purifying contaminated water from heavy metals, which represents an efficient, environmentally friendly and low-cost treatment technology (Forni, Chen et al. 2001). By doing so, it can be concluded that *A. pinnata* will serve as a target for wastewater treatment in easily managed industrial. As demonstrated in this study, the removal efficiency demonstrated by healthy pre-weighed *A. pinnata* was relatively higher for lead and copper and lower for cadmium (Ali, Abbas et al. 2020).

1.5 Azolla production

Azolla can be produced with the help of the formation of an artificial water body, preferably in the shade of Silpaulin Sheet, protocol steps cab be outlined as:

1- As a first step, a hole of 2m x 2m x 0.2m should be drilled .

2- This hole is covered with plastic glue to prevent the roots of neighboring trees from penetrating the celluline plate which is spread over the plastic gunnies.

4- Then about 10-15 kg of fertile soil, sifted, are uniformly distributed on the cellulose /celluline sheet.

5- Next we pour clay made from 2 kg of cow dung and 30 grams of superphosphate in 10 liters of water onto the sheet .

6- Then we pour more water till the water level is about 10 cm. Then, about 500 g to 1 kg of fresh and pure Azolla culture are inoculated into the new pit.

7- Azolla will grow very quickly and fill the hole in 10-15 days and we can harvest approximately 600g of Azolla per day after that .

8- A mixture consisting of 20 grams of superphosphate and about 1 kg of cow dung should be added 1 time in 5 days.

9- This is done to keep Azolla in the rapid multiplier production stage and to maintain a daily yield of 500g / pit .

10- A mixture of micro-nutrients containing other necessary elements such as magnesium, iron, copper, sulfur, etc. can also be added at weekly intervals to improve the mineral content of Azolla (Gourkhede 2021).

Figure 1.16: Azolla appears floating in an agricultural basin.

1.5.1 Create an artificial Azolla pond

To create an Azolla cultivation pond, select an area that's partially shaded because Azolla needs 30% of sunlight, since too much sunlight will damage the plant. The area under the tree is preferred. If you decide to grow Azolla on a large scale, you can make small 3/5 concrete ponds otherwise you can make the basin any size you want. Dig the soil for the pond and level the soil afterwards. Spread the plastic sheet around the ground to prevent water loss. Make sure that the depth of the pond is at least 20 cm.

Add some soil uniformly to the plastic sheet in the pond. For a 2m x 2m pond, add 10-15 kg of soil. Azolla needs phosphorous to grow well, you can use superphosphate with cow dung. Cow dung increases the available nutrients. Use cow dung 4-5 days old. Next, fill the pond with water to a level of about 10 cm. This will allow the short path of the Azolla to float freely and then leave the pond for 2 to 3 days for the ingredients to settle. After 2-3 days, add the Azolla culture to the pool by rubbing the Azolla gently with your hand. Helps split Azolla into smaller chunks for faster doubling . Two weeks after the start of harvest. Pond size of 2m x 2m, you can harvest 1 kg Azolla every day.

Figure 1.17: Azolla plastic basins with distinctive designs.

1.5.2 Important tips to grow Azolla

- Azolla rapidly grows so maintain Azolla biomass 300 g – 350 g/sq. Meter harvest daily to avoid overcrowding.
- Add once in 5 days mixture of Super Phosphate, and cow dung also add mixture containing magnesium, iron, copper, Sulfur etc. at weekly intervals to enhance the mineral content of Azolla.
- Replace 25 to 30% old water with fresh water, once in 10 days; it helps to prevent nitrogen build up in the pond.
- Replace the water and soil completely, at least once every six months and then add fresh Azolla seeds.
- Maintain the water level at least 10 cm so that the Azolla root does not grow in the soil by keeping the roots afloat and making them easy to harvest.
- The harvested Azolla is washed well to remove the dirt and smell of cow dung, then feed it to the animals.

2. Problems Statement and Objectives

2.1 Research problem

This research provides practical solutions to the problem of the high cost of animal feed, its scarcity and the lack of local production in sufficient quantities for the number of livestock in the Palestinian territories, which led to a decrease in the economic return and in many cases. . Farmers' losses due to the massive increase in the prices of traditional animal feeds used on their farms. This has led to the reluctance of many of them to raise livestock and their tendency to work in other sectors such as construction, trade and work in the occupied Palestinian territories, which has led to a sharp decline in the number of livestock in general. Including birds, fish and livestock, this drop pushed prices up. Products. Animals in general and the consumer were affected as a result of this increase. The Palestinian farmer also suffers from the problem of high prices of chemical fertilizers, which leads him to leave the rain-fed crops without fertilization, which reduces their production and profit from them. On the other hand, the Palestinian territories suffer from severe scarcity of fresh water resources, waste and misuse of sanitation sources, and are wasted without the slightest exploitation.

In this research, we try to provide integrated practical solutions to these two problems, by exploiting drinking water sources, fish ponds, and sewage water after being well treated, and cultivating Azolla structures to produce organic fertilizers and protein-rich animal feeds in an easy, simple, safe and environmental way.

2.2 Objectives

The main objectives is studying the possibility of cultivating Azolla plant in Palestine.

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The Specific objectives are:

1. Measuring the effect of water type on the growth rate of the Azolla plant .
2. Measuring the effect of the type of manure on the growth rate of Azolla.
3. Study of the effect of temperature on the growth rate of Azolla plants.
4. To Compare between the growth rate of Azolla and duck weed plants.
5. To Study the possibility of fertilization and biological movement of the water and soil of Azolla ponds using certain types of fish.
6. Studying the effect of sterilizing the organic fertilizer added to Azolla ponds on the growth rate of algae and fungi inside them, and consequently the growth rate of Azolla.
7. Ensure that the different farm animals such as fish, birds, sheep, goats, cows, calves and others are palatable to eat the Azolla plant.

2.3 Study theory

1. There is a close relationship between the type of water and the growth rate of Azolla.
2. There is a relationship between the type of manure used and the growth rate of Azolla.
3. There is a relationship between the temperature in the water and the Azolla growth rate and productivity.
4. The growth rate of the Azolla plant is less than the growth rate of the water lentil plant.
5. There is a relationship between light intensity and Azolla plant growth rate.
6. Sterilizing the organic fertilizer before adding it to the Azolla ponds contributes to preventing the growth of algae and fungi.

7. Some types of herbivorous fish can be raised inside the Azolla ponds to provide them with the necessary elements for their growth and to dispense with fertilizer.
8. Most farm animals like to eat Azolla.

2.4 Research questions

1. Is it possible to grow the Azolla plant in the climate and environment of Palestine?
2. What is the effect of the type of water on which the Azolla plant is grown on its growth rate?
3. What is the effect of the type of organic fertilizer used to fertilize the Azolla plant on its growth rate?
4. What is the effect of temperature on the growth rate of Azolla plant in the Palestinian environment?
5. What is the difference between the growth rate of the Azolla plant and the water lentil plant?
6. Is it possible to fertilize Azolla ponds using certain types of fish?
7. What is the effect of sterilizing the organic fertilizer added to Azolla ponds on the growth rate of algae and fungi inside them?
8. Do farm animals accept Azolla as part of their daily feed?

Chapter 2

Materials and Methods

This study was conducted in a greenhouse mainly at Al Majd village, Hebron, Palestine. Some experiments was conducted at Beit Al-Rush village Hebron, Palestine.

The main study site was at Al Majd (312850N 345701E), Al Majd is a village located eighteen kilometers south-west of Hebron, it is located 475 m above sea level, the average annual rainfall is 436 mm, the average annual temperature is 16 C and the average annual humidity is 61%. The climate is hot and dry in summer, cold with moderate temperatures in winter.

2.1 Collection of plant Material

Azolla pinata was collected from Jenin on 27 October 2020. The plant was transferred during the night hours in a plastic bag that was wet from Jenin to Hebron; to keep it as moist as possible. On the safety of the plant, the plant is supposed to be planted as soon as possible and it is not desirable to delay it. On the third day, a little water was added to maintain the plant's moisture. Then, a group of wooden basins were prepared with an area of one square meter for each basin and planted the plant in one of these basins.

2.2 Chemicals , Reagents and Tools

Table 2.1: Reagents and Chemicals used in cultivation of *Azolla pinnata*

	Component	**Quantity**
Chemical materials	Copper sulphate	300 g
	Hydraulic acid	1/2 L
	Table salt	300 g.
	Balanced industrial fertilizer	3 Kg
	Superphosphate	500 g
	phosphoric acid	1/2 L
Several types of natural fertilizers	Cow manure	10 kg
	Sheep manure	10 kg
	Bird manure	10 kg
	Vermicompost	10 kg
Different types of water	Fish water	50 L
	Treated wastewater	50 L
	drinking water	50 L
100% Methanol	-	1 Bottle

Table 2.2: Tools used in cultivation of *Azolla pinnata*

	Component	Quantity
pH and Conductivity meter	-	1
Mass balance	-	1
Thin mesh	-	1 meters square
Analytical Balance	-	1
Spatula	-	1
Tissue Paper	-	1
Centrifuge	-	1
Spectrophotometer	-	1
2 mL Eppendorf Tubes	-	Bag
1L Beakers	-	5
500 ml Bottles	-	8
Microscope	-	1
An area of 30 square meters inside a greenhouse	-	30 square meters
Small agricultural ponds (Different Sizes)	-	22
Black agricultural plastic	-	(9) 150*150

2.3 Culturing experiments

2.3.1 Growing Azolla without soil with the use of different types of organic fertilizers or nutrient solutions

This experiment is conducted to determine the effect of using red agricultural soil in Azolla ponds when fertilizing with different types of organic fertilizers. It took place between 10th June 2021 and 15th July 2021.

Five groups of basins, each group consisting of 3 basins (5*3). It was prepared with an area of half square meter for each basin and a depth of 20 cm. Soak 1/2 kg of each of the following types of manure (cow, cattle, and birds) in 3 liters of water. Then the soaked manure was sterilized for each of the three types using a sulfate compound. Copper was added at a concentration of 2 g/liter and this is the was added to 1 m^2 basin. Table 2.1 describe the used treatments and the fertilizer types and concentrations.

Table 2.3: Treatments that were used for Azolla culturing.

Treatment	Description of fertilizer
1	Cow manure drenched with a concentration of one liter of the soaked solution/basin.
2	Cattle manure drenched at a concentration of 1 liter of the soaked/basin.
3	bird manure (broiler chickens) at a concentration of one liter from the soaked/basin.
4	Vermicompost liquid at a concentration of (1/4) kg/basin
5	Chemical nutrient solution.

After that, each of the aforementioned ponds was fertilized with phosphorous element by adding phosphoric acid to each pond at a concentration of 2 g/basin. Then Azolla was added to the ponds in the previous five treatments to the same extent, and the resulting changes were observed in each of the ponds during a month.

Meanwhile, the basins were moved on a daily basis. A suitable shade was provided inside the greenhouse by spraying the shale solution over the plastic to reduce the intensity of lighting. After that, all the formal and physical changes that appeared on the Azolla plant were recorded and observed in terms of shape, mass, color and others characteristics. Biomass of Azolla in all the basins of the fourth and fifth groups was measured at regular intervals (every 5 days once).

2.3.2 Measuring the maximum biomass capacity of Azolla plants that can be obtained from planting a square meter basin

This experiment is conducted to measure the maximum biomass of Azolla after planting in the previous treatments in section 2.3.1. And it was extended from 25th November to 25th December 2020.

3 wooden basins are equipped with an area of 1 square meter for each sink and a height of 20 cm. Then water was added at a height of 18 cm in each basin. Then, the basins was fertilized with well-dried and fermented animal manure at a concentration of 0.5 kg/m^2. After that 1 kg of Azolla was added, this is followed by moving the basin every day, usually in the morning or in the evening and sometimes at noon.

Then the ponds were fertilized with phosphorous element using phosphoric acid at a concentration of 5 g/m^2, while continuing to check the temperature of the ponds almost continuously. After a month of planting, all the Azolla in the pond was extracted and dried in a well shaded place away from the wind for a period After 24 hours, and the maximum mass of Azolla that can be obtained from a square meter was measured.

2.3.3 Comparing the growth rate of Azolla with the growth rate of water lentil

This experiment is conducted to compare the growth rates of Azolla and water lentil. It was extended from 30th November to 30th December 2020.

Water lentils was brought from the wastewater treatment plant of Al-Aroub camp. The lentils was placed in a water basin fertilized with dried animal manure at a concentration of 0.5 kg/m^2. The area of the basin was a square meters and height of 20 cm, and the basin was placed inside a greenhouse. After five days, 3 small basins with an area of half a square meter were prepared and 50 g of water lentils were placed inside them, with the addition of 1.5 g superphosphate in each of them in order to help in plant proliferation and reduce the percentage of toxins. The water was kept stirring inside the basins, and after two weeks of planting the basins. The average amount of water lentils in the three basins was measured, while continuing to stir the basin periodically after that. Then two wooden basins with an area of one square meter were prepared, the inside of each was covered by a plastic cover and soil (thickness 3 cm). The two basins were placed in the same environment as Azolla (temperature, humidity, water level, soil type, organic fertilizer, fertilization with superphosphate and intensity of light). 50 g of water lentil was added in the first basin with an area of 1 m^2, another 50 g of Azolla was added to a second basin with an area of 1 m^2, and in a third basin 25 g of Azolla and 25 g of water lentils were added together. The 3 basins were stirred a daily basis once or twice. The results was recorded on a daily basis.

Finally, two groups of basins (area of half a square meter) were prepared, each group consisting of 3 replicates, the first group was planted with 50 g of Azolla, the second group was planted with 50 g of water lentils. After that, both groups were monitored and data was recorded in terms of growth rate. Growth rate was measured by calculating the mass of the plant in each basin after drying for 6 hours in the shady place.

2.3.4 Studying the effect of water quality on the growth of Azolla

50 liters of treated wastewater were brought in a secondary treatment from the Wadi Al-Aroub wastewater treatment plant. The treated wastewater was placed in three basins placed in the greenhouse, with an area of half a square meter for each basin and a height of 20 cm (Group A). Then, 60 liters of water from fish ponds containing tilapia were brought from the neighboring of Beit Al-Rush village and placed in the three basins with an area of half a square meter for each basin (Group B). Another 3 basins were prepared and filled with drinking water (from the well), soil was added without any fertilizer at a height of 3 cm (Group C). After that, readings were taken for pH and the electro conductivity EC, while continuing to stir the water and soil in the group C once or twice a day. Growth of Azolla was monitored and biomass was measured for the three groups. This experiment was conducted during the winter of 2020, from the 12th of January and for a month.

2.3.5 Cultivating Azolla using fish water in different ways

This experiment took place in a greenhouse at Beit Al-Rush village from 24th December 2020 to 5th February 2021. Four wooden basins were built with an area of one square meter for each basin and a height of 20 cm. In the first basin, Fish water was added to a height of 15 cm, then red agricultural soil was also added with a thickness of 3 cm. As for the second basin, drinking water was added to a height of 15 cm, this was followed by the addition of red agricultural soil with a height of 3 cm and half a kilo of organic non-dry fertilizer. In third basin, only fish water was added. And in the fourth basins only drinking water was added. After that, 100 g of Azolla was added in each basin, while continuing to turn the basins on a regular basis. After 20 days of cultivating Azolla, for the first time mass of Azolla in the basins was measured, then the process was repeated after 20 days twice (day 40 and 60), also the pH was measured in each of the previous basin.

2.3.6 Measuring the impact of moving Azolla basins in which soil is incorporated into the construction of their agricultural environment

Two sets of basins were made, each group consisting of 3 basins, each basin area is half a square meter and a height of 20 cm, and the basins were placed inside a greenhouse to provide the appropriate environment for the growth of Azolla, and agricultural soil with a height of 1-2 cm was placed inside each basin in both groups.

Preparation of the liquid organic fertilizer soak, by dissolving 10 kg of fertilizer in 30 liters of water, then the organic fertilizer soak was sterilized with copper sulfate compound at a concentration of 5 g/liter of the fertilizer soaked volume for two days, after two days of sterilizing the liquid organic fertilizer soak the fertilizer was added To the basins of the two groups at a concentration of one-third of a liter of liquid fertilizer infusion/basin, then superphosphate was added at a concentration of 5 g/m^2 to all the basins in both groups. After that, Azolla (50 g/basin) was added to each basin in both groups, and then the ponds was monitored on a daily basis. While leaving the basins of the first group without moving, and moving the basins of the second group, with the addition of a soaked organic fertilizer and superphosphate every two to three weeks for both groups as usual, and the experiment was repeated in the summer inside the same greenhouse to see if the effect of stirring in the summer is similar to its effect in the winter or slightly different.

2.3.7 Study the effect of temperature on the growth rate of the Azolla

This experiment was conducted to evaluate the growth rate of Azolla inside and outside the greenhouse. Two groups of basins were prepared, each group consisting of 3 basins with an area of half a square meter for each basin, where the first group was placed inside the greenhouse (plastic) and the second group outside the greenhouse. After that, water and fertilizer (chemical fertilizer and phosphoric acid) and sterilizers were added at the same time and the same quantity (25 g/m^3) for both group. Then, both groups were shaded with a black agricultural net (50% shade), with the lighting ratio adjusted to be close to the farthest extent, then the growth of both groups was monitored on a daily basis, pond mass was measured every 5 days in both groups. This experiment was extended for a month, from 20th March to 20th April 2021.

2.3.8 Comparison between the growth rate of Azolla in summer and winter

3 wooden basins lined with nylon were built, the area of each basin is 1 square meter and the height of each is 20 cm. After a day, a layer of soil with a height of 2 cm was placed in each basin, then each basin was filled with water to a height of 15 cm. After two days it was fertilized with dry manure, manure was sterilized with copper sulfate at a concentration of 5 g/m^2. After three days 100 g of Azolla was placed in each basins, with continuous stirring on a daily basis of water and soil at a rate of once a day, and after 6 days 5 g of superphosphate was added. After 10 days and after

ensuring the acclimatization of the Azolla in the new basins, the quantities placed inside each pond were adjusted to become 100 g inside each basin.

Green Azolla biomass was measured every 5 days (note: in this experiment we were measuring the mass of all the plants in the basin, not half of the area, after siege and drying in the shade for 7 hours). The experiment was continued in the winter cycle for 30 days, it was repeated in summer 1st June 2021, with the same previous steps and quantities, and data were recorded every 5 days.

2.3.9 Study the effect of covering Azolla basins planted outside the greenhouse with a plastic cover during the winter and cold days to protect them from frost

Two sets of basins were prepared, each group consisting of three basins, the basin area is 0.5 m^2, the height of the basin is 20 cm, and the basins were placed in the same place where it is exposed to the same weather conditions and external environmental influences. Then they were filled with water at a height of 15 cm, after that red agricultural soil was added to with a depth of 3 cm. Organic fertilizer was prepared by soaking 2 kg of dry organic fertilizer in 6 liters of water at a concentration of 5 g/l, it was soaked for 48 hours. Soaked organic fertilizer was added to the two groups and after 24 hours, phosphoric acid was added to each basin at a concentration of 2 g/basin or 5 g/m^2, after 48 hours of fertilization, 50 g of Azolla was added. The first group was covered with a transparent plastic cover every night from three in the afternoon until nine in the morning. Then the cover is removed until three o'clock in the afternoon (during very cold days, it is left covered constantly), and the previous step was repeated daily for a month. As for the basins of the second group, they were left without any coverage.

For the first week after planting Azolla, it was left without any measurement in order to help Azolla to adapt to the new environment. Observations were recorded daily, were morphological changes and Azolla biomass was recorded. Azolla in the two basins (200 g) in each tank was reset and experiment was repeated 3 more times.

2.3.10 Studying the effect of sterilizing Azolla ponds on preventing the growth of algae and fungi

Two sets of basins were prepared, each group consists of four basins, the area of each basin is half a square meter and the height of each basin is 20 cm. The two groups were placed at the same environment and at the same conditions (outside the plastic house due to the lack of space), basins were filled with water to a height of 15 cm in each basin. Then, a non-sterile green bovine organic fertilizer was added to the first group basins with a concentration of a quarter of a kilo of organic fertilizer/m^2 (drenched manure). At the same time, sterilized green bovine organic manure with copper sulfate at a concentration of 5 g/l was added to the second group's basins at a concentration of a quarter of a kilo of fertilizer/m^2 (the fertilizer soaked), noting that the fertilizer was sterilized for a full 48 hours. The water was sterilized in the first group using copper sulfate at a concentration of 5 g/m^2, and then phosphoric acid was added at a concentration of 5 g/m^2 to the first and second group ponds. 50 g of Azolla was added to each of the two groups, and after that the ponds were moved once and on a daily basis, with growth control for algae, molds or fungi in sterilized Azolla tanks in two different ways. Rate of Azolla biomass production was recorded weekly basis for 3 consecutive weeks.

2.3.11 A preliminary experiment of self-fertilizing Azolla ponds through tilapia or catfish inside the tank

This experiment was conducted to test the possibility of raising fish in Azolla ponds, it was performed from the 1st of May 2021.

3 basins were initially prepared, each basin area of 2 m^2, where the three basins were filled with pure water from an agricultural water pool, then 3 cm thick layer of agricultural red soil was placed in the first three basins only. Later, on the next day two tilapia fish were placed in the first basin for composting and stirring, noting that the fish was kept in part of the basin so as not to devastate Azolla plants at the beginning of its reproduction. Then, after 10 days, the two fish were released, and the second basin was fertilized with organic and chemical fertilizer, it was soaked with a quarter kilo of animal manure/m^2, then 5 cm^3 phosphoric acid/m^2 was added. The third pond was left without any fertilization. After two days 800 g of Azolla was placed in each of the three ponds, and it was left for 5 days to adapt. The growth rate of Azolla at each of the three ponds was monitored for 20 days, and after 5 days of cultivation, the amount of 800 g of Azolla was set in each pond. Then, only 800 g of Azolla was returned to the basins, and after 5 days, its mass was calculated again, this was repeated for another time.

2.3.12 Measuring the effect of the type of fertilizer on the growth of Azolla plants

Seven groups of agricultural basins were prepared, and each group consisted of 3 agricultural basins, the area of each basin was 0.5 m^2. Different types of fertilizers were added to each basin, Table 2.2 shows the type of fertilizers used at this experiment.

Table 2.4: Treatments that were used to study the effect of fertilizers type on Azolla growth.

Groups	Type of fertilizer
1	Green bovine manure
2	Sheep manure
3	Chicken manure
4	Fish water
5	Vermicompost, 0.5 kg/m^2
6	Nutrient solution 5 g NPK
7	Control, no fertilizer is added

All types of organic fertilizer were dried separately for a week in the sun except for Vermicompost. Red agricultural soil was added to the first four groups, then the seven groups were filled with water at a height of 12 cm except the fourth group.

Compost was added to the seven groups, it was prepared by dissolving one kilogram of dried organic fertilizer in 3 liters of water (the first three groups), as for the fourth group in which fish fertilizer will be used, there is no need to make a fertilizer soak because its fertilizer is dissolved in the water. For the fifth group, compost was added as it is, while the sixth group was fertilized with solid chemical fertilizer NPK a concentration of 5 g/m^2. The organic fertilizer soaked for the first three groups was made for each group separately at a concentration of 1 kg of dried organic fertilizer/3 liters of water, by dissolving fertilizer in water for two consecutive days, then the resulting fertilizer soak was sterilized with copper sulfate compound at a concentration of 5 g/l, then the soaking was left for 48 hours, then the chemical nutrient solution was added to the sixth group ponds at a concentration of 5 g/m^2, and then fish water was added for the basins of the fourth group as an alternative to water and manure, then vermicompost was added at a concentration of half a kg/m^2.

All basins were transferred to the greenhouse, and it was shaded with the shading to protect the Azolla from direct sunlight and to decrease the appearance of algae, Azolla amount adjusted by adding 50 g of Azolla to each basin. After one week, phosphoric acid was added to all groups. Soil in the basins of the seven groups was continuously stirred on a daily basis once a day, and Azolla growth was monitored on a daily basis. Azolla mass was recorded in all groups once every 5 days and for a period of 20 days.

2.3.13 Measuring the amount of water consumed by Azolla per month

3 basins of Azolla were prepared, the area of the each basin was 2 m^2, with a height of 20 cm, water was added to all basins, to a height of 18 cm. After that, the basins were planted with Azolla at a rate of 1 kg/basin. Then, the soil was added to the basins and fertilized with a drenched organic fertilizer at a concentration of a quarter of a kilo of organic fertilizer/m^2. Phosphoric acid was added to each basin at a concentration of 3 g/m^2, pond water was stirred on a daily basis, and Azolla was harvested continuously. Data for water loss was recorded on a weekly basis, and this experiment was conducted for one month from 1^{st} July to 1^{st} August 2021.

2.3.14 Study the effect of growing Azolla at different heights of water and its effect on Azolla growth rate

4 groups of basins were prepared, each group consisting of 3 basins, the basin area is 0.5 m^2 and its height was 20 cm. Then all basins were filled with water to a height of 20 cm. After that, red agricultural soil was added to a height of 2-3 cm and fertilized as usual with a sterilized organic manure at a concentration of a quarter kg/ m^2. The height of the water in the second group was reduced to 15 cm, and in the third group it was reduced to 10 cm, and in the fourth group’s basins it reduced to a height of 5 cm. after that, Azolla was planted in all basins, by adding 50 g in each pond, Azolla was left for one week to adapt to the new environment, then the amount of Azolla in each pond was reset to 50 g. After 15 days of cultivation, Azolla mass was recorded in the four groups, this is repeated every 5 days.

2.4 Experimental Design and Statistical Analysis

All experiments were arranged in a Completely Randomized Design (CRD) and significant difference was tested by the analysis of treatment means. Descriptive statistic was used to analyses for means and draw the graphs.

Chapter 3

Results and Discussion

3.1 Growing Azolla without soil by using different types of organic fertilizers or nutrient solutions

The growth rate of the Azolla in all basins of the first group (basins was fertilized with dried sterilized cow manure fertilizer without using agricultural soil), the second group (basins was fertilized with dried and sterilized cattle manure without using red agricultural soil) and the third group (basins was fertilized with dried and sterilized bird manure without using agricultural soil) was very weak, and the plant began to turn yellow at the end of the first week (figure 3.1). The size of the plant was smaller compared to the size of the original plant at the time of planting. Moreover, the plant was about to die permanently by the end of the month, and its color was clearly yellow (figure 3.2).

Figure 3.1: Azolla basin planted organically using fermented animal manure and red agricultural soil. the plantlets looks green and healthy at the beginning of the experiment, after one week the plants began to be weak and its color turned to yellow.

Figure 3.2: Azolla after one week of planting.
Azolla looks weak and its color turned to yellow, Azolla was grown using only animal manure and no agricultural soil.

The main reason and the only logical explanation for the appearance of the aforementioned symptoms in each of the basins of the first, second and third groups is mainly due to the lack of many nutrients needed by the Azolla to proliferate, it clear that these nutrients was not found in the added organic fertilizer.

On the other hand, the growth rate of the Azolla in all the basins of the fourth group, which was fertilized with vermicompost and without using agricultural soil was normal. The color of the plant was green to bright green, and the size of the plant was in the normal ratios. In my opinion, the reason why the basins of this group are distinguished from the basins of the rest of the groups that vermicompost contains all the nutrients needed by Azolla to grow naturally. Moreover, the growth rate of the Azolla plants in the fourth group's basins was slightly greater than the growth rate of the Azolla plant in the fifth group's basins, but the cost of planting using vermicompost was slightly higher compared to fertilizing with chemical nutrient solutions. This does not mean that fertilizing with vermicompost is always better than fertilizing with nutrient solutions in terms of the rate of food mass production since there are different types of nutritional solutions that we have not tried.

Figure 3.3: Azolla grown organically using only vermicompost (fourth group).
The growth rate of Azolla plant was higher than the other groups, the plants color was green to bright green. The plants proliferated in normal pattern and the size of it was in the normal ratios.

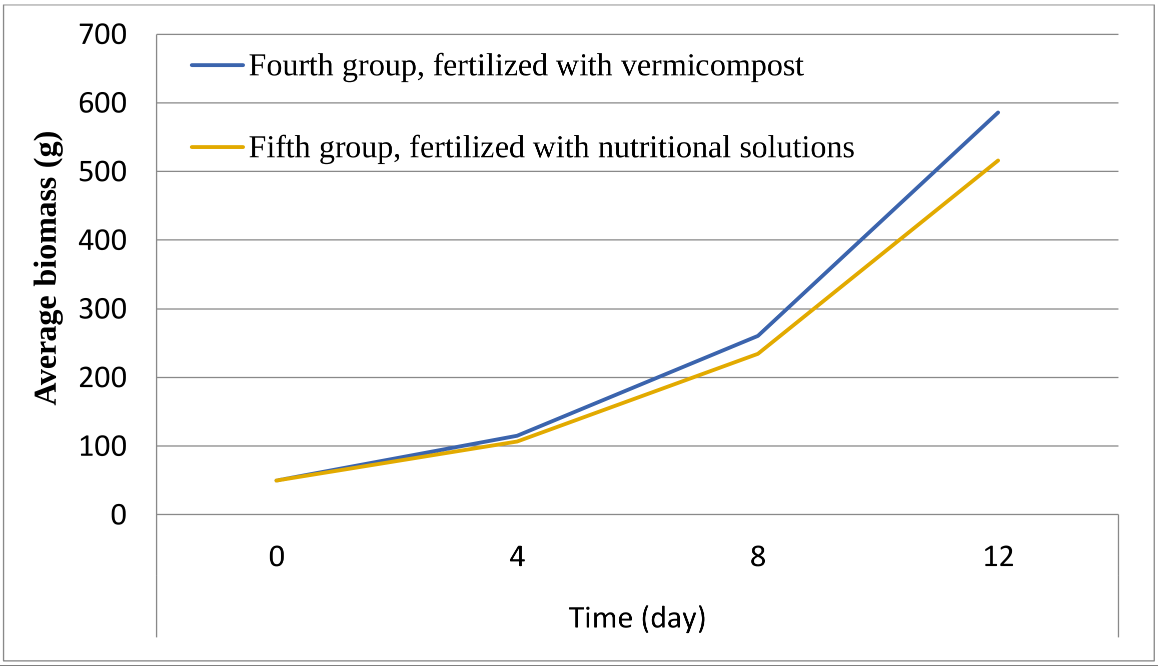

Figure 3.4: Comparison of the growth rate of Azolla fertilized using chemical fertilizer or vermicompost.

Figure 3.4 shows the difference in the growth rate between the basins of each of the fourth and fifth groups, and results show that fertilizing with vermicompost are slightly better than fertilizing with the nutrient solution in terms of production. The multiplication rate in the fourth group basins is approximately equal to 2.25, While the doubling rate in the fifth group basins was approximately 2.12.

From the results it can be concluded that it is not possible to cultivate Azolla without using soil except in two cases, firstly when Azolla ponds is fertilized with nutrient solution that make up for the lack of basic elements found in the soil, which cannot be obtained from cow, livestock or bird manure. Secondly, when Azolla basins fertilized with vermicompost, because it contains all the nutrients needed by Azolla to grow normally.

Therefore and to overcome the problem of poor nutrients on Azolla basins, it is advised to perform the following: firstly, add soil to Azolla basins when fertilizing with dried and sterilized animal manure. Secondly, in areas that do not contain soil or suffer from soil salinity or lack of arable soil, it is possible to find an alternative for the success of Azolla cultivation. But one of the drawbacks to this that fertilizer is more expensive compared to other types of fertilizer unless it is produced locally.

3.2 Measuring the maximum biomass capacity of Azolla plants that can be obtained from planting a square meter basin

Azolla biomass from the cultivated area (1 m^2) was harvested and dried in the shade for 24 hours, and it was approximately 800 g. initial Azolla mass was 1 kg/basin. The duplication time was every 4 days, and so Azolla has a 7.5 dupling time for one month. According to these results it is estimated that one square meter basin produces 6 kg of biomass, and so it can be estimated that one dunum can produce 6 tons of Azolla biomass.

Results showed that there was an increase in the thickness of the layer of Azolla that covers the surface of the basins was 3 or 4 times the normal thickness. The reason for this is that the entire area of the basin is filled with Azolla, which made it accumulate in the form of a thick layer that can reach in some to 5 cm.

Figure 3.5: Azolla cultivated on 1 m^2 basin, the basin was fertilized with well-dried and fermented animal manure at a concentration of 0.5 kg/m^2. Azolla increases in thickness and leaf size when harvesting is delayed.

While monitoring the temperature of the basin in the morning, evening and noon, it is noticed that there is a difference between the temperature of the water adjacent to the thick layer of Azolla and the temperature of the bottom water that don't have thick layer of Azolla. The reason for this difference in temperature is that Azolla works as a thermal insulator that reduces the heat exchange process between the water of the basin and outside it, this help basins to retain water temperature for longer period especially in winter.

It is observed that aging has its effect on Azolla plants, the roots began to change their color to brown and then black. The possibility of root mold infections in old and large plants increases if the plant is left for a long time without reducing it. The plant start to weaken and dies physiologically. Although the basins was not sterilized as a result of using decomposed and dry organic fertilizer for a long time, no fungi or algae appeared. However, other aquariums show poor growth of algae in a late period. This is mainly due to the controlled blocking of sun light using an agricultural net used for shading (blocks the light by 50%) and also because of the use of decomposed dried organic fertilizer that is free of moisture, this make it free from several pathogens.

Based on the results of the second experiment, in order to achieve a higher production rate, in the event that there is not enough space for cultivation, it is better to reduce the Azolla continuously when the basin is full and not to wait for the thickness of the Azolla layers to increase. Because filling the basin significantly and increasing the thickness of the Azolla layer reduces the growth rate, as it is theoretically expected to produce one kilogram of Azolla within a month if sufficient space is provided for it, or if the basin is constantly reduced, an amount equivalent to 15 kg in ideal conditions. But due to space constraints and the lack of relief from the basin, and despite the high thickness of the Azolla a lot, this did not compensate for the shortfall in space, as the result of cultivating 1 kg after a whole month .

Figure 3.6: Azolla harvest after initial drying in the shade for 7 hours.

It is possible to benefit from increasing the thickness of the Azolla layer in heating the plant in the winter, as it is preferable to reduce the harvest as much as possible. Thickness can help in the cold weather and it would contribute to the heating and protection of Azolla from death, especially in the basins that are grown outside a greenhouse.

It is preferable to harvest Azolla from the basins continuously, in order to produce new young plants that can be used later as a seedlings. Also, this can help in the protection of Azolla farm, since it can be affected by microorganisms such as mold. Moreover, aging of Azolla plants decrease its numbers, usually the death stage of

Azolla begins after two to three months from cultivation, so continuous harvest and dilution can restore its youth.

According to our observations on some of the experiments that we conducted in the summer, the thickness of Azolla layers left in the basin for a period of one month without dilution is significantly greater than their thickness during the winter season. Therefore Azolla growth rate was higher and so produced higher biomass. This can be contributed to the high temperatures and light intensity in summer days compared to winter.

Estimation of Azolla productivity during a month

As shown, Azolla multiplies at a very fast rate, doubling once every 3-5 days in ideal conditions and at a rate of 7-12 days in cold climate conditions. Azolla can complete 7-8 agricultural cycles, if the appropriate conditions for its cultivation are available such as temperature, fertilizer and shade.

Agricultural monthly cycle is calculated by dividing a month/multiplication time and it equals (30/4 = 7.5). The mass of one square meter of green Azolla after harvesting and drying in the shade for approximately 8 hours is 1600 gm.

Assuming the cultivation of one dunum (1000 m^2) of land from the Azolla plant, it is necessary to harvest half of that area (1000/2=500 m^2) every 4 days approximately. Since the mass of each square meter of Azolla after drying in the shade for 8 hours is approximately equal to 1600 grams. The total mass expected to be harvested from the Azolla plant grown on an area of one dunam can be calculated by the following equation :

Productivity of a half a dunum = productivity per square meter * area harvested

= 1.6 kg * 500 = 800 kg.

To calculate the productivity of a certain area during a month (30 days), we find the product of multiplying the productivity during one cycle by the number of cycles per month, so the result becomes as in the following equation:

Productivity of a certain area during a month (30) =

productivity of one cycle (4 days) * number of cycles during a month (7.5)

= 6000 kg = 6 tons .

3.3 Comparing the growth rate of Azolla with the growth rate of water lentil (duckweed)

Growth rate of Azolla and water lentil was calculated after two weeks of culturing, the results show higher growth for both plants. Morphologically, both plants show vigorous growth, and the color was green. Figure 3.6 shows the average biomass of the Azolla and water lentil after 4 days and after two weeks of culturing, the average biomass of Azolla was 18.2 g and for water lentil it was 23.7 g/day.

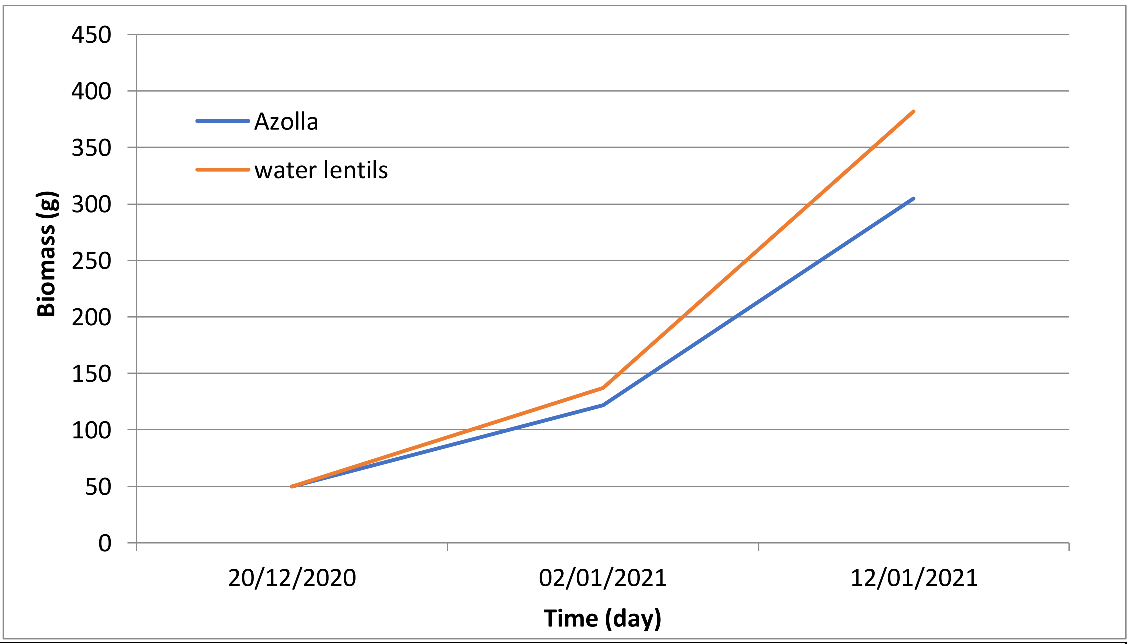

Figure 3.7: Azolla and water lentil biomass every 10 days after culturing.

Water lentils covered the entire basin after 35 days under winter conditions. When growing water lentils and Azolla outside, the growth rate of Azolla is superior to the growth rate of water lentils. While in summer, water lentils outperform Azolla in growth rate.

Figure 3.8: Growing Azolla and water lentil in the same agricultural basin.

The growth rate of water lentils was faster than the growth rate of the Azolla plant, especially in the warm environment and in moderate and relatively warmer temperatures. In very cold and cold temperatures, the rate of reproduction of Azolla is slightly greater than the rate of reproduction of water lentils. This can be attributed to the effect of culturing Azolla and water lentil at the same basin, water lentil plant had covered most of the basin in the warm seasons, and vice versa in very cold seasons (figure 3.8).

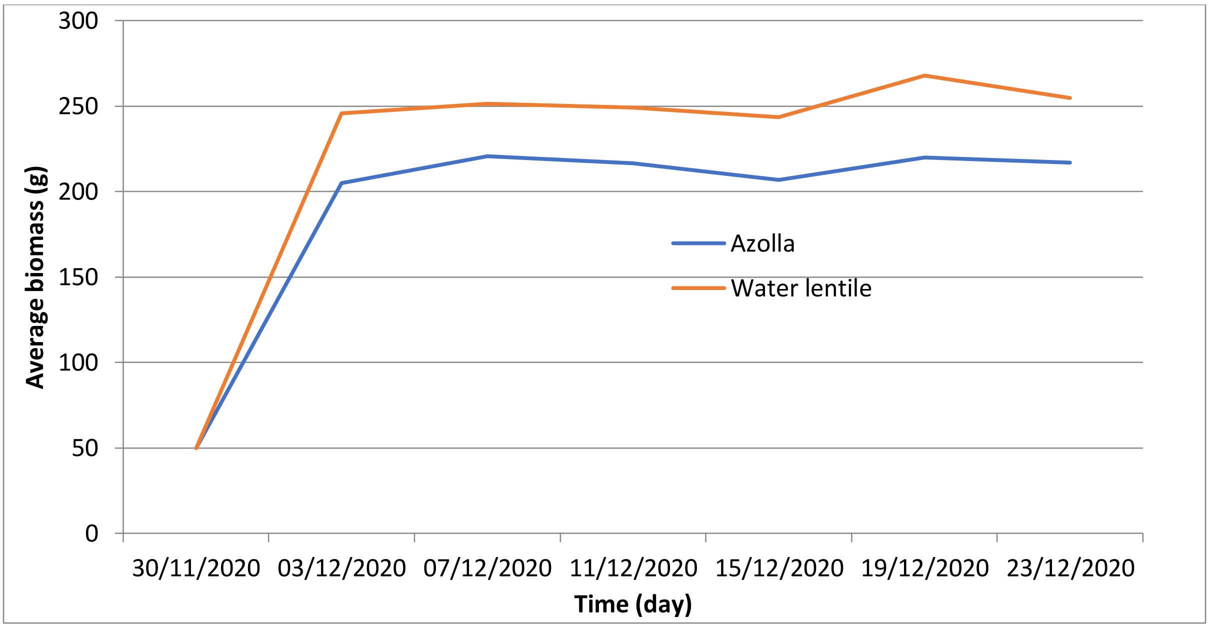

Figure 3.9: Average biomass of Azolla and water lentil every 4 days after culturing. Both plants are cultivated in the same basin.

The water lentil plant can be grown in an environment very similar to the environment of the Azolla, this may be because both are tropical aquatic plants and live in the same conditions and the same environment. It is possible to grow the Azolla plant and the water lentil plant together in the same basin, because of the great similarity between the environmental and nutritional needs of each of these two plants (figure 3.9).

3.4 Studying the effect of water quality on the growth of Azolla

In this experiment wastewater has been used to cultivate Azolla. Wastewater was produced from tertiary wastewater treatment plant. Table 3.1 shows the results for pH values before planting Azolla. It clear that the water has a moderate pH values and so it is similar to normal water.

Table 3.1: The average pH in the three basins before planting the ponds.

Group	A	B	C
pH	7.3	7.1	6.3

The growth rate of Azolla plants in group B basins that were grown on fish ponds water was greater than the growth rate of Azolla plants in the rest of the basins, as it was followed immediately by group A basins that were grown on treated wastewater, and then followed by group C basins that contain drinking water and soil without using organic fertilizer (figure 3.10).

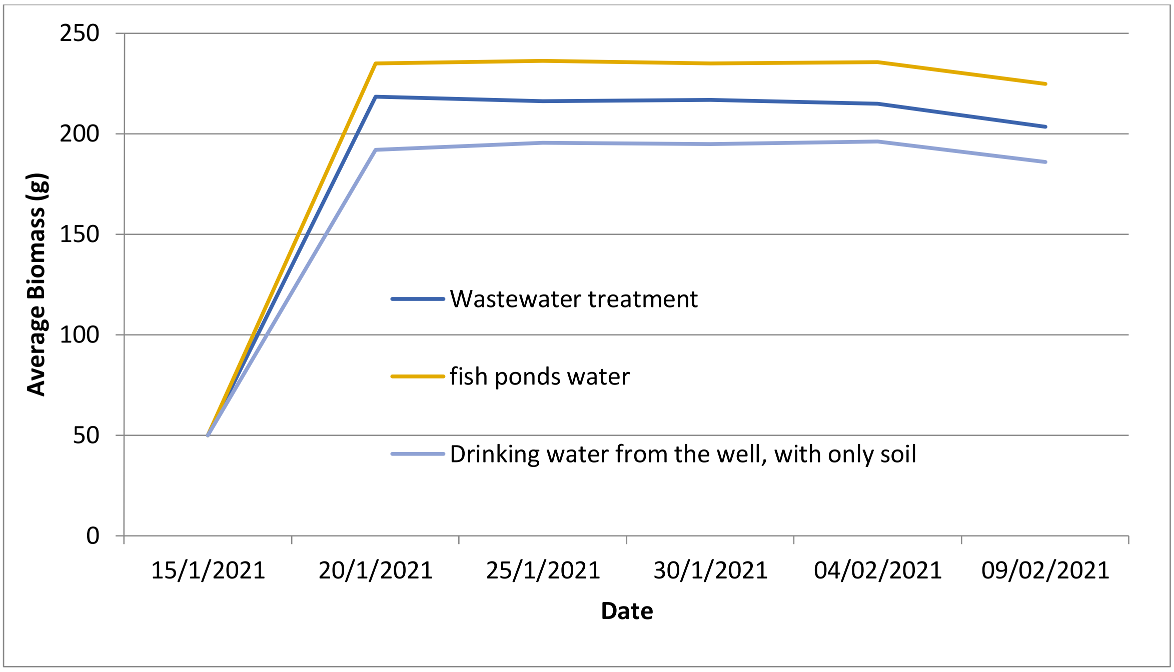

Figure 3.10: Average Azolla biomass cultivated on three treatments (waste water G1, fish waster G2 and drinking water G3). Data were recorded every 5 days.

Results show that Azolla has fast growth rate at the first two days, initially 50 g of Azolla was cultured in the treatments, on the second days it has an average biomass of 218.3 g for G1, 235 g for G2 and 192 g for G3. After three days of culturing Azolla, the plant in the three treatments has a stationary growth and after 6 days the plant biomass started to decrease. This may be attributed to the cold weather in this period of winter. Table 3.2 shows the average Azolla biomass after 5 days of culturing in the three treatments.

Table 3.2: Average Azolla biomass every 5 days.

Group	Average biomass (g)
Wastewater treatment (G1)	213.8
Fish ponds water (G2)	233.3
Drinking water from the well, with only soil (G3)	192.8

It can be concluded, that water from fish farming can be used to grow Azolla, or even grow Azolla on top of the fish basins themselves, and then use it as animal feed or as green fertilizer. Fish ponds with Azolla on top may have higher temperature as described above. Higher water temperature will make the water more suitable for fish growth and Aquaculture i.e. tilapia fish aquaculture. Fish water contains important nitrogenous fish wastes, which is very rich in ammonia and many other nutrients, most notably phosphorous. The treated wastewater can be used as a second choose for Azolla cultivation. Green biomass of Azolla grown on waste water cannot be used, nor used as food for animals, since Azolla in this case contains a high percentage of heavy and toxic elements that harm both animals and humans. The produced Azolla in this case could be used in the production of energy or biofuels, because the Azolla plant contains a high percentage of starch that can be converted into sugar that can be fermented to produce ethanol.

3.5 Cultivating Azolla using fish water in different ways

Results was recorded for the first time after 20 days of culturing, then it was repeated twice on a 20 days interval, data are shown in table 3.3. Results show that the first treatment has the highest biomass followed by the second , third and fourth treatments (figure 3.11, 3.12 , 3.13, 3.14 respectively). This clearly indicate that fish water has high effect on the growth of Azolla.

Table 3.3: The growth rates of Azolla grown on fish water.

	Average Azolla biomass (g)			
Date	First basin Fish water and red agricultural soil	Second basin Drinking water and red agricultural soil	Third basin Fish water	Fourth basin Drinking water
15/1/2021	826	589	557	294
5/2/2021	835	583	549	295
25/2/2021	833	591	551	288

It was observed that algae appeared on the basins, this may be due to the failure of covering the basins or because of the partial shading. This can expose algae and Azolla to the direct sunlight, which play a pivotal and main role in the emergence, growth and spread of algae on the surface of the basin and the competition of Azolla in its food and sticking to its roots later.

It can be concluded that the water of fish farming is considered excellent source for breeding Azolla, especially if it is mixed with agricultural soil. Mixed fish water and soil are good combination because the soil contains elements in a good proportions that Azolla needs to maintain its growth, these elements are not present in the water of fish basins, or they are present in weak concentrations (figure 3.12) .

Figure 3.11: Azolla in fish ponds containing agricultural soil.

Figure 3.12: Azolla cultivated in a drinking water basin containing agricultural soil.

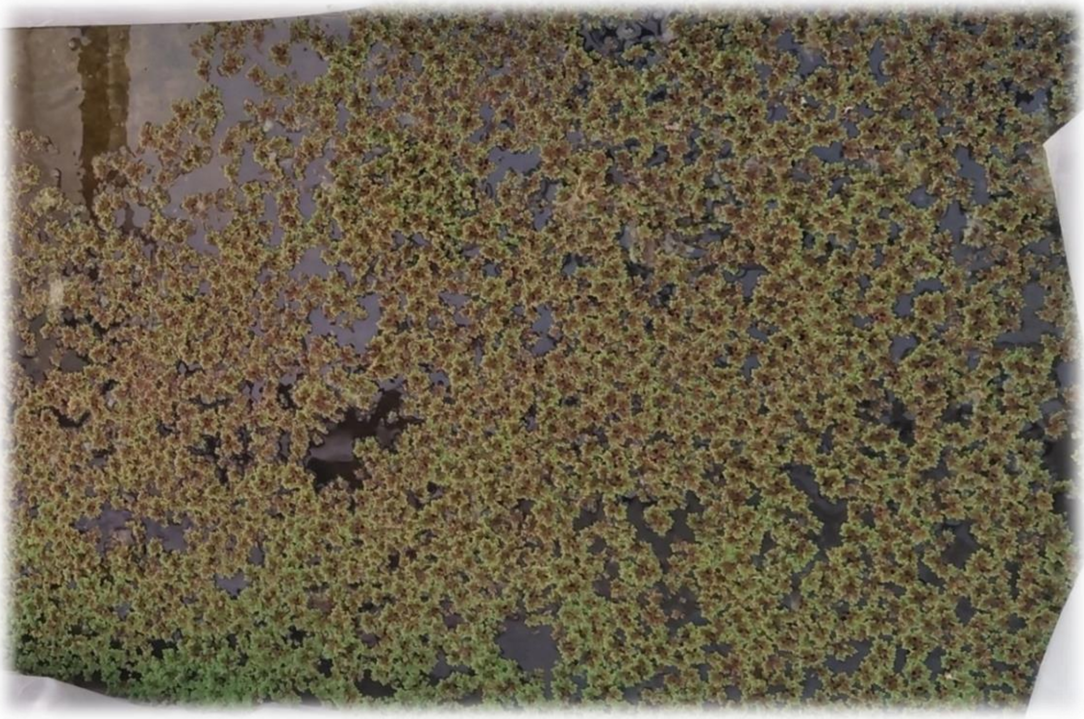

Figure 3.13: Azolla cultivated in fish ponds and without agricultural soil.

Figure 3.14: Azolla was grown on drinking water only and without agricultural soil.

Accordingly, it is strongly advised that Azolla plants is cultivated side by side next to the fish basins, and so Azolla can be used as food for fish, and we can use the water of the fish ponds to fertilize the Azolla basins. It is preferable also to add red agricultural soil to water of fish when constructing Azolla cultivation basins. It is also recommended to grow Azolla in separate basins from intensive fish farming basins, If the goal of fish farming is to fish production. However, if the goal of adding fish is fertilization, we recommend reducing the number of fish as much as possible for the fear of increasing the concentration of ammonia in the water and this may cause plant burning.

3.6 Measuring the impact of disturbing Azolla basins in which soil is incorporated into the construction of their agricultural environment

This experiment was conducted twice in winter and in summer, the aim was to study the effect of disturbing water basins on the growth rate of Azolla. Figure 3.15 show the results of the two treatments, it is clear that disturbing or stirring the water in Azolla basin have an effect on the growth rate of plants as group 2 has produced higher biomass.

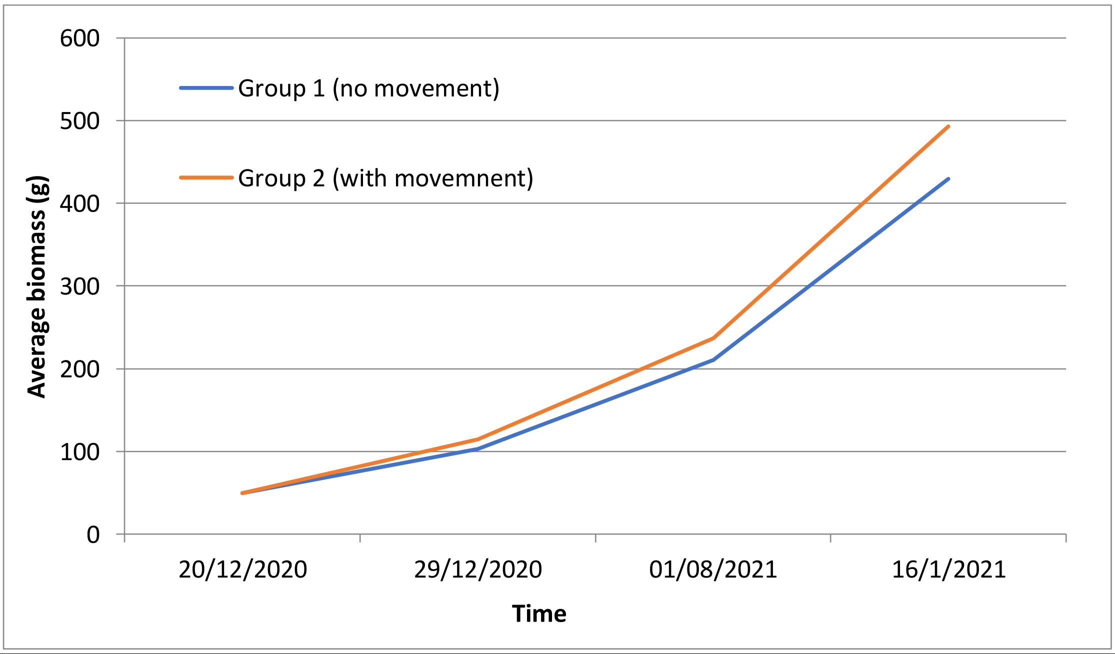

Figure 3.15: Average Azolla biomass in winter. Azolla plants was cultivated by two ways, without moving the water in the basin G1, and with moving the water in the basin G2.

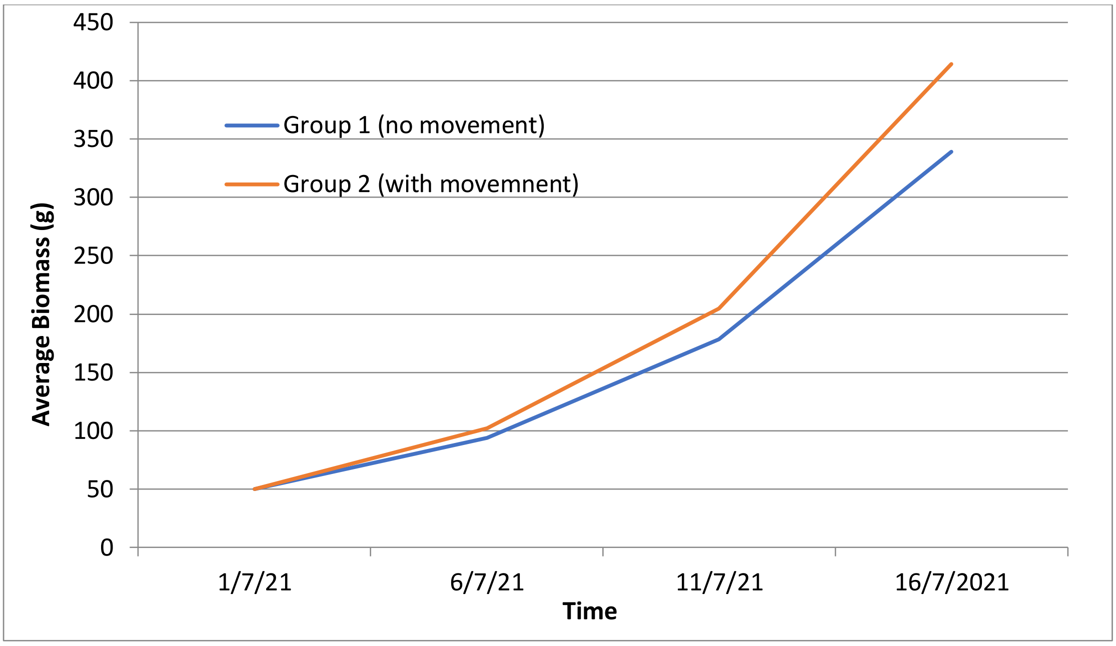

Figure 3.16: Average Azolla biomass in summer. Azolla plants was cultivated by two ways, without disturbing (stirring) the water in the basin G1, and with disturbing (stirring) the water in the basin G2.

During the months of summer, it was clearly observed that the mass of the Azolla in the basins that were stirred was approximately 10% greater than the mass of the Azolla inside the basins that were never stirred. During the winter months, the biomass produced from the basins of the second group was 5% greater than the biomass produced from the basins of the first group .

This unambiguously shows the importance of stirring the water in the basins, which increases Azolla biomass in summer and in winter. Moreover, stirring the water has a role in preventing the occurrence of soil rot in the added soil in the basins. Another and essential reason is that moving the basins soil contributes to the dissolution of some of the essential elements in the soil and thus facilitates its access to the roots of Azolla. Essential elements play an important role in maintaining of Azolla health and this accelerates the rate of growth and reproduction.

It was observed that in the cold months Azolla can grow and multiply almost naturally, in the presence or absence of water movement. The plant grow in normal rate and no diseases appeared, the color did not change, and no algae or any molds appeared. Also it did not suffer from the problem of insects significantly during the winter period. The growth rate in winter was very low compared to summer and this was as a result of low temperatures and not as a result of lack of movement. The effect of no movement on the growth rate of Azolla has clearly appeared in the winter days, one explanation that Azolla plants condensed on itself to form a thick layer, and thus it was protecting itself by forming a thermal insulator for the aquarium, thus protecting its environment and maintaining water temperature.

One of the main problems that has appeared especially in summer is the growth of molds and algae. Soil contains several microorganisms such as bacteria, molds and algae, and they will increase if the soil is left unmoved for a long time. These microorganisms grow due to the suitable temperature and lack of movement of basins (figure 3.17).

Figure 3.17: The appearance of algae, bacterial and mold (green slimy masses) on the surface of Azolla ponds in which agricultural soils has not been moved.

3.7 Study the effect of temperature on the growth rate of the Azolla

It was observed that the Azolla plant can grow inside and outside the greenhouse, but the growth rate of the Azolla planted outside the greenhouse is slower compared to the plant grown inside the greenhouse, especially during the cold months (figure 3.19 ,3.20). This is due to the low temperatures outside the greenhouse compared to inside it. Figure 3.18 show the results obtained by monitoring the plants for two months, by the end of the experiment the plants cultivated inside the greenhouse has an average biomass equal to 497.3 g and plants cultivated outside the greenhouse has an average biomass of 429.3 g, statistically this considered to be not significant.

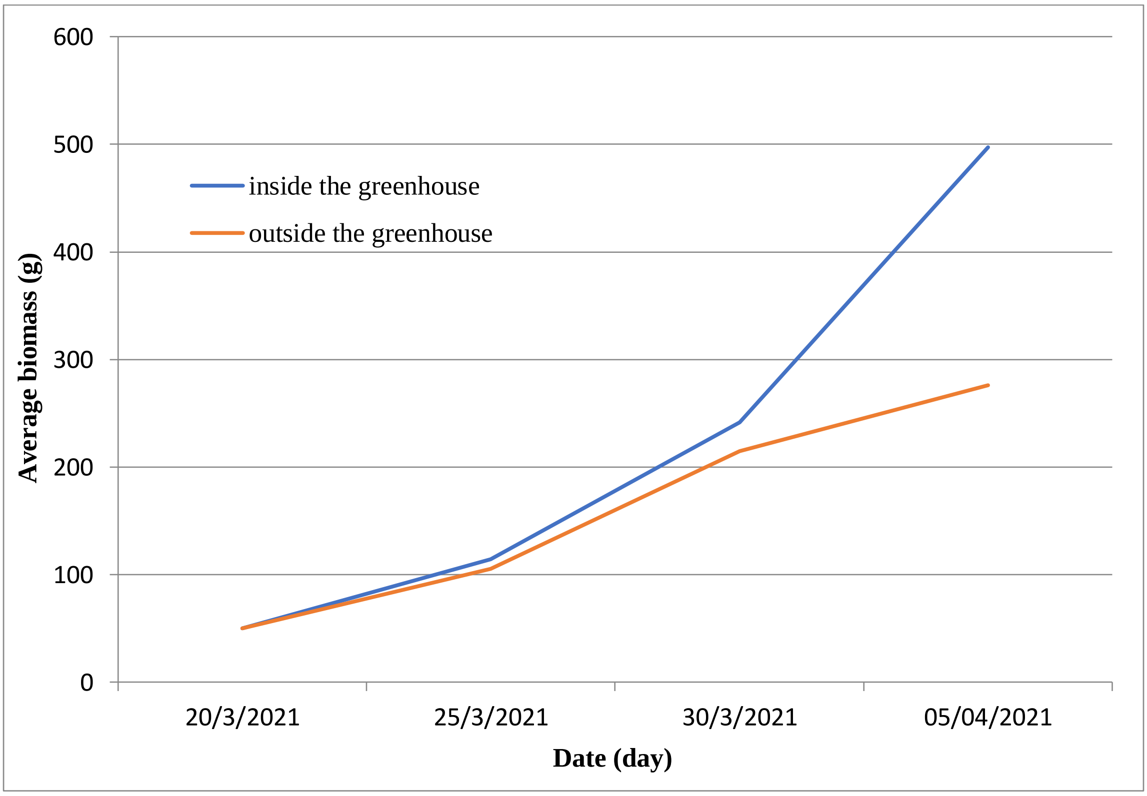

Figure 3.18: Average Azolla biomass cultivated inside and outside a greenhouse.

Figure 3.19: Azolla planted in a plastic house has excellent growth.

Figure 3.20: Azolla planted outside the greenhouse.

Based on the results, the ratio between the growth rate of Azolla inside the greenhouse and the growth rate of Azolla outside the greenhouse during 20 days is 1:2, during 3 times of doubling. It is recommended to grow Azolla inside the greenhouse, because the temperatures are suitable for the growth and multiplication of Azolla, since it is a tropical plant and favor high temperatures, which range between 20 – 35 C The result of this experiment recommend to plant Azolla in the Jordan valley region. The high temperature in the Jordan valley will be suitable to grow Azolla outside greenhouses. In conclusion, the results are consistent with the results of Hechler and Dawson (1995) and the results of Tuan and Thuyet (1979), the average temperature of both studies was around 32 C and this is in regard to location.

3.8 Comparison between the growth rate of Azolla in summer and winter

Azolla plant can be grown in summer and winter, but the growth rate of the Azolla plant in the summer is almost twice as high than in the winter, and it can reach four times according to the climate of the region, where the Azolla plant doubles in the cold months once every week to 15 days. While it doubles in summer once every 3-5 days. The reason for this is the difference in temperatures between summer and winter. Table 3.4 shows the results in summer and in winter; average biomass was measured every 5 days from the beginning of the experiment.

Table 3.4: The growth rates of Azolla grown in summer and in winter every 5 days.

Season	average biomass (g)				
	1	2	3	4	5
winter	100	152.3	231.3	346.3	532.3
summer	100	223.7	491.3	992.0	2013.3

Figure 3.21: Keeping Azolla ponds warm in winter by delaying harvesting.

It has been noticed that disturbing the water of Azolla basin in the summer contributes to controlling the temperature of the basin and reducing the temperature of the water due to the lower temperature of the mud in the summer compared to the temperature of the water. So, it is recommended to increase the thickness of the soil layer inside the basin and so it can contribute effectively to control the temperature in summer and winter. Moreover, results from the winter cycle validate our results in section 3.6 on the effect of Azolla layers and not moving the water and soil in basins. As it is indicated Azolla thick layers plays as a thermal insulator and so it contributes by stabilizing basins temperature.

It has been noticed that the color of Azolla plants has been changed in the summer and this can be due to the direct exposure to sunlight for long period and high intensity. Long exposure to sunlight can initiate the transformation of the green chlorophyll pigments into the red exothianin pigment (ref). In order to control the appearance of the red color, partial shading must be provided for the Azolla plants to protect it from direct sunlight. Also, algae blooms has been appeared due to the high temperatures and sunlight availability, it is recommended to sterilize the basins and avoid adding new organic fertilizer and protect it from exposure to direct sunlight.

The results are similar to the conclusions of El-Hakeem et al (2000), where it is clear that seasonal variations between summer and winter affects the growth rate of Azolla.

3.9 Study the effect of covering Azolla basins planted outside the greenhouse with a plastic cover during the winter and cold days to protect them from frost

Figure 3.22 shows the average biomass of Azolla in both treatments, the average biomass of the basins that is covered during the night was higher than the basins that was not covered. It can be concluded that the growth rate of the first group's basins was greater than the growth rate of the basins of the second.

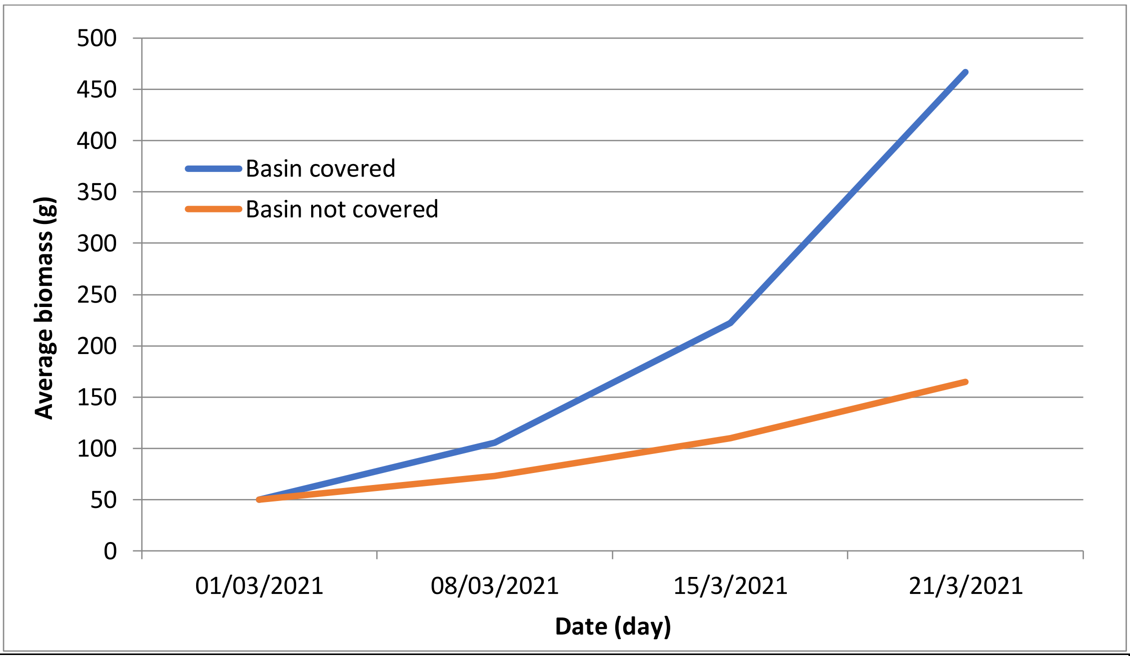

Figure 3.22: Average Azolla biomass cultivated in basins in which the first group was covered during the night and the second group was not covered.

Data was collected on a weekly basis from the day of cultivation, by the end of the experiment Azolla basins that is covered had an average biomass of 211 g/day, and the basins that is not covered had an average biomass of 175 g/day.

After three weeks, Azolla had been harvested from all the basins, Azolla was dried in the shade for 7 hours before finding its mass, in order to ensure the disposal of water from Azolla leaves and to ensure its infiltration.The average biomass of Azolla harvested from the basins of the first group was greater than the average biomass that was harvested from the second group basins. The average air mass resulting from the basins of the first group was 467 g, while the average biomass from the second group basins was much less than that, where it was 165 g. The ratio between the results of both groups after a week (the relative doubling time) was approximately equal to (1:1.5), and it is worth noting that this amount doubles in a manner ascending and unstable with time depending on the changes of other environmental factors such as heat, light, frost, etc. The logical explanation for these results is caused by the transparent plastic cover that preserves the heat of the basins and prevents it from leaking out, in other words, it limits the process of heat exchange between the basins' water and the cold external environment.

Figure 3.23: basins of the first treatment, which has been covered by plastic cover overnight.

It can be concluded that covering Azolla basins overnight during the cold months increases the rate of biomass production from these basins. Therefore, we recommend covering the Azolla basins planted outside during the cold months and frost days and during the night hours, due to the low temperature usually during the evening hours compared to the hours of day when the sun is shining.

3.10 Studying the effect of sterilizing Azolla ponds on preventing the growth of algae and fungi

This experiment was conducted to evaluate the effect of sterilizing the fertilizers used to cultivate Azolla. A non-sterile green bovine organic fertilizer was added to the first group basins at a concentration of 250 g/m^2 (drenched manure). In the second treatment, sterilized green bovine organic manure was added to basins, it was mixed with copper sulfate at a concentration of 5 g/l , the fertilizer was sterilized for a full 48 hours. Results Azolla biomass was recorded for three weeks, data are shown in table 3.5

Table 3.5: The average biomass of Azolla for three weeks of cultivation.

	Average biomass (g)		
Group name	20/3/21	27/3/21	04/04/2021
First group (non-sterile organic fertilizer)	50	75	97
Second group (sterile organic fertilizer)	50	83.25	132.5

Results show that the growth rate and biomass production of Azolla in the basins of the second group is greater than the basins of the first group. The average Azolla biomass after three weeks was 74 g for the first group and 88.6 g for the second group. It has been observed the rapid emergence and growth of algae, it grows rapidly and formed masses that start to affect the growth rate of Azolla (figure 3.24). Algae compete with Azolla for food, light and space, as it turns around Azolla roots penetrate them and get food directly from them, thus the plant's ability to absorb nutrients decreases and its growth decreases accordingly.

The growth of green algae was observed in relative abundance in all the basins of the first group that were sterilized with copper sulfate compound at a concentration of 5 g/m^2, after adding the green organic fertilizer to the basin. While in contrast the growth of algae was observed on only one basin of the second group. The explanation for this is simply due to the fact that the concentration of copper sulfate in the drenched organic fertilizer is greater than its concentration when it is added directly in Azolla basin, and thus the sterilization effect increases when sterilizing the organic fertilizer drenched directly for 48 hours compared to adding the compound directly to the basin.

Therefore, it is recommended to sterilize the fertilizer soak before adding it to the aquarium water. The sterilization method is by adding 5 g of copper sulfate to 1 liter of the organic fertilizer soaked for at least two days, then it is added to the basins.

Figure 3.24: Weak growth of Azolla in ponds that are fertilized with non-sterile organic fertilizer and the appearance of algae on them.

3.11 A preliminary experiment of self-fertilizing Azolla ponds through tilapia or catfish inside the tank

This experiment was conducted to evaluate the possibility of raising fish in Azolla basins. For the first treatment two tilapia fishes were added to the basins, in the second treatment 250 g of animal manure/m^2 was added followed by the addition of 5 cm3 of phosphoric acid/m^2, the third treatment was a control and so it was left without any fertilizers.

It was noticed that the Azolla grew in the first and second aquariums normally and without any problems during the first three weeks. At the end of the fourth week, it was noticed a clear change in the color of in the first aquarium water, this is may be due to the high concentration of ammonia resulting from fish residues, and it was reduced by adding new fresh water to the aquarium. Azolla's growth rate in the first basin increased with the beginning of the third week due to the high percentage of fertilizer coming from fish waste, results are shown in table 3.6.

Table 3.6: The effect of using fish to fertilize Azolla ponds on their reproduction rate.

		Average biomass (g)		
Treatments	planted quantity	20/5/2021	25/5/2021	1/6/2021
Group 1 fertilizing with fish	800	1441	1522	1759
Group 2 Fertilizing with a nutrient solution	800	1616	1750	1762
Group 3 without fertilizing	800	1194	1156	1163

It is possible to self-fertilize Azolla ponds by raising tilapia or catfish inside Azolla basins at the rate of one or two fish per square meter (fish density), but taking into account the continuous addition of water. Fish density describes the number of fish within one square meter, and it should be low to maintain Azolla growth rate since the purpose of the fish is not for breeding but for fertilizing and continuous stirring of water and soil in the basins. If the fish density exceeds a certain limit, the fish and Azolla number will decline leading finally to death, this happened as a result of the high concentration of ammonia in the water .

In summary, fish fertilizer is considered relatively excellent for the growth of the Azolla because of its richness in organic materials and mineral elements dissolved in water such as phosphorous, potassium, nitrogen, carbon and other materials necessary for the growth of Azolla.

3.12 Measuring the effect of the type of fertilizer on the growth of Azolla plants

Results was collected every 5 day interval. As shown in figure 3.25, the growth rate of Azolla in the basins of the fifth group that were fertilized organically with vermicompost was considered as the highest among all groups, followed by the sixth group that were fertilized chemically with nutrient solution. Fourth group (fertilized organically with water from fish basins), first group (organically fertilized with cow manure), and the third group (organically fertilized with pigeon and chicken manure) has relatively similar growth rates. Azolla cultivated at the basins of the second group that were organically fertilized with sheep manure showed lower growth rate and biomass production. The seventh group has declined with time, its color has changed to yellow and after 20 days Azolla plant died.

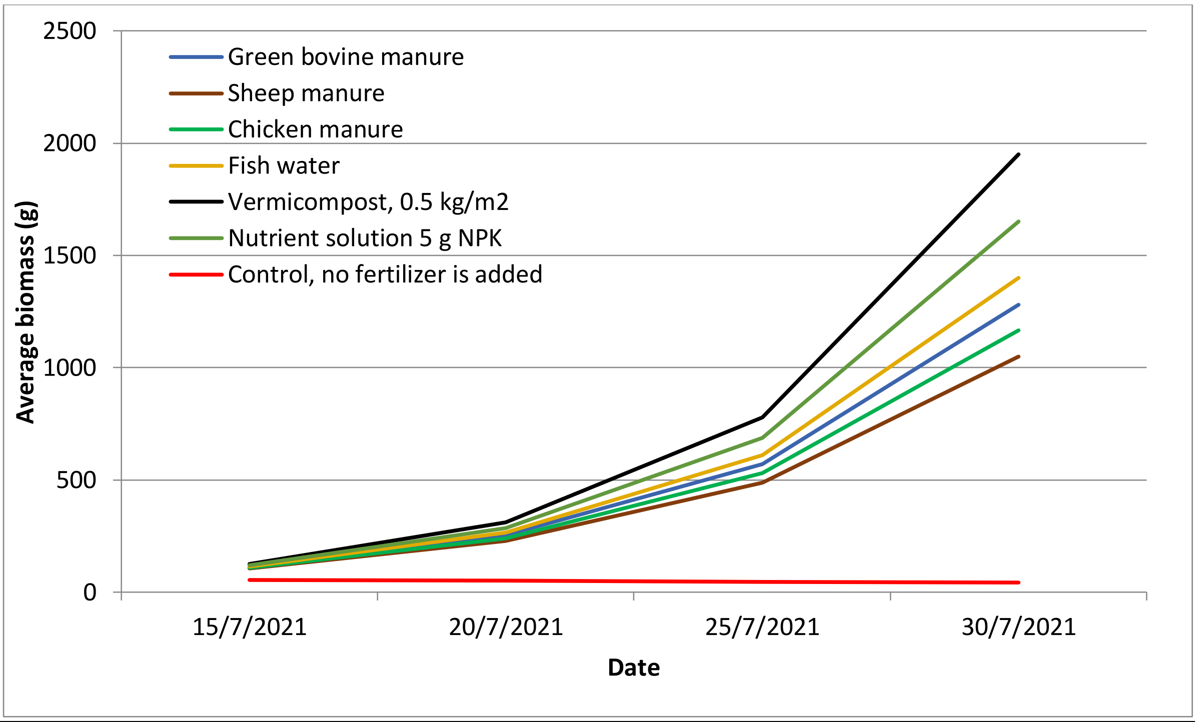

Figure 3.25: Average Azolla biomass cultivated in 7 different groups.

Figure 3.26: Arranging and preparing Azolla basins before planting.

Azolla can be fertilized by using agricultural soil and drenched organic fertilizer only and without any additives of any substance or chemical element, because agricultural soil and drenched organic fertilizer contain the most important nutrients that the Azolla plant needs in order to grow naturally without any symptoms of disease. Also, Azolla can be fertilized using agricultural soil and fish water only, because the water of fish basins contains fish wastes that is rich in nutrients important for plant growth, especially phosphorous, which is dissolved in water and is easily absorbed by the roots of the Azolla plant. Moreover, Azolla can be grown and fertilized in several main ways such as, chemical fertilization and natural organic fertilization using drenched organic fertilizers of animal origin, vermicompost or water from fish basins.

Vermicompost at a concentration of 0.5 kg/m^2 has the highest and best growth rate (1951 g) compared to other six treatments. This can be due to the fact that vermicompost contain several macro- and micronutrients at different ration that aid in plant growth and also work as soil conditioner. Secondly, the nutrient solution 5 g NPK has achieved the second best results, Azolla growth rate was 1652 g. This also can be contributed to the high concentrations of nutrients, nitrogen, phosphorus and potassium. Manure treatments (fish, bovine, chicken and sheep) was closely related to each other, fish manure was the highest, the average biomass of the previous treatments was 1401, 1282, 1167 and 1049 g respectively. Also, these results can be contributed to the effect of high content of organic and inorganic components found in the manure.

3.13 Measuring the amount of water consumed by Azolla per month

Table 3.7: Average water consumption in Azolla ponds.

	Average water loss (cm)		
Basin	after ten days	20 days later	30 days later
First	4	8	12
Second	4	8	12
Third	4	8	12

According to the results shown in Table 3.7, the water level in Azolla basins decreased by an average of 12 cm per month, and it is known that this amount is variable and not fixed, as it varies with different seasons and the difference in the prevailing climate in the region in terms of heat, humidity, wind and others.

Depending on the this results, the amount of water needed by one dunum of Azolla per month can calculate.

3.14 Study the effect of growing Azolla at different heights of water and its effect on Azolla growth rate

The results that we obtained from the experiment showed that the average biomass generated from the basins of the first group (586.3 g) is higher than the average biomass generated from the basins of the rest of the groups. This was expected, and the reasonable explanation of this is that during the hot months the higher the water level in the basins, the greater the plant can control the temperature around it. Moreover, high temperatures of the basin water negatively affects the rate of absorption of elements by the roots of the plant.

Figure 3.27: preparation of Azolla basins before starting the experiments.

The color of Azolla in the basins of the third and fourth group changed with time to become reddish brown and is directly proportional to the decrease in water depth within the basins of the two groups. On the contrary, the basins of the first group did not show any apparent change in color and external shape. The explanation for this is simply due to the transformation of the green pigment chlorophyll into the red pigment exothianin.

The water temperature in the basins of the first group was relatively moderate compared to the water in the basins of the second group. The water temperature in the basins of the second group is lower than the water in the basins of the third group, as well as the water in the basins of the third group is less than the temperature of the water in the basins of the fourth group. There is a clear inverse relationship between the temperature of the water in the basin on the one hand and height and depth of the water in the basin. Less height of water in the basin, cause higher water temperature, which negatively affects the rate and efficiency of absorption by the roots of the Azolla plant of the mineral elements necessary for the process of growth, reproduction and photosynthesis, which significantly affects the rate of the biomass.

The results indicate a high correlation between the depth of water and the growth rate of Azolla, and this can be explained by the effect of temperature fluctuation and the ability of water to maintain a suitable temperature. Vermaat et al (2000) and Santamaria (2002) has explained that shallow water induces winds that has direct and indirect effect on Azolla and it can cause mechanical damage and so reduce the growth rates.

Figure 3.28: Azolla was planted on water with a depth of less than 5 cm.

3.15 Case study: Interviews with Successful stories of Azolla cultivation

These interviews was conducted by the researcher either by phone, Email or using social media such Facebook.

In the first section, the researcher interviewed success stories in the Arab worlds.

First interview: Dr. Asaad Al doq

Dr. Asaad is a distinguished researcher and farmer living in the Arab Republic of Egypt. He is considered one of the most successful farmers who introduced Azolla into the feed of their animals. He has achieved this by carful studied experiments and at different proportions, this lead him to a successful application on the ground without recording any health problems or a decline in productivity.

We asked Dr. Asaad About the animal species that he tried to feed it Azolla and in what proportions. He replied that he used Azolla to feed broiler and laying chickens, especially municipal laying hens. Also he tried to feed Azolla to sheep's, cows and ducks. He assured us that it is not possible to rely on Azolla completely in breeding, but we enter it at different proportions that vary according to the type of animal.

We asked him about the percentages that succeeded with him effectively, he answered that he introduced Azolla into the feed of broiler birds at a rate of 20-25%, and for laying hens, he assured us that it was at a rate of 25% and he obtained the best results in the case of laying hens. we asked him also about introducing Azolla to sheep's, and he told us that he makes a customized fodder mixture consisting of 50% ground yellow corn, 10% bran and 40% dried Azolla, in addition to calcium carbonate (1.5 kg/100 kg), table salt (1 kg/100 kg) and mineral salts (a quarter of kg/100 kg). When we asked him about raising cows, he answered that he uses Azolla in its green form, not dried, as in the case of sheep. After filtering Azolla from the water for 48 hours in the shade, he makes a mixture consist of 40% dried Azolla, 50% yellow corn and 10% bran, then he presents it to the cows.

In the case of raising ducks, he replied that he provides ducks with half their needs of traditional fodder and puts 70% of Azolla. He assured that Azolla saved him large sums of money. In addition, the animals appeared in excellent health and with more abundant milk production in both sheep's and cows.

Second interview: Engineer Abdul Hakim Al Shanwani

Eng. Abdul Hakim is an Egyptian agricultural engineer and an expert in the field of Azolla cultivation and animal husbandry. He had made several realistic practical experiments during which he used Azolla in raising different animals such as laying hens, ducks, sheep, cows, quails, fish and others.

We asked him about his experiences in raising municipal laying hens, he replied that he introduced Azolla into the daily feed of the birds, and the results were excellent. The Mixture was 30% dried bread crack, 30% rice crack, 20% dry Azolla, 20% green Azolla, calcium carbonate (35 g/ kg), table salt (60 g/kg), and vitamins (1 g/kg feed). He said that if the breeder wants to increase the quality of the feed composition, he can use one of the concentrated feed types instead of using broken bread.

When we asked him about the production of laying hens on which he conducted the experiment, he assured us that he had conducted the experiment on 17 hens, and within 3 days their production rate was 37 eggs.

Third interview: Dr. Ali Zughayer

Dr. Ali is from Syria and he used Azolla in the feed of his animals such as hens, sheeps, and cows. and when we asked him about his experience with laying hens, he told us that laying hens up to a month old eat one kilogram from the fodder and after reaching the age of one month, he begins to introduce the Azolla with the feed freely and separate from the traditional feed, i.e. without mixing, and it continued in this case until the age of 120 days until the chick begins to lay eggs and then continues to put the Azolla with the feed freely. He noticed that the consumption of the chick from the traditional feed decreased from 135 g/day to 95 g/day, and the rest was compensated by Azolla, meaning that it saved almost 35% of the traditional fodder.

We asked him also about his experience with broiler chickens, he replied saying that one bird up to the age of 40 days consumes 4 kg of feed and its weight is 2100 g. And when he started to add Azolla from the age of 20 freely, the weight of the bird increased by an average of one kg during the same period of time (that is, within 40 days) to become 3100 g. Also, we asked him about the sheep, he replied saying that he provides them with a regular meal of fodder in the morning and a meal of green Azolla in the evening. In regard to cows, he told us that he uses 6 kg of Azolla feed and 4 kg of conventional feed. By doing this he saves 4 kg per cow on a daily basis, and he noticed an increase in milk fat, density and production rate.

Fourth interview: Dr. Hassan Bilal, this interview is a summary of an interview of Dr. Hassan on the YouTube channel of engineer Hassan al-Amiri. The researcher also contacted al-Amiri for other information's.

Dr. Hassan is a poultry nutrition specialist, and he used Azolla to feed different types of animals on his farm. He confirmed that he tried to introduce Azolla with different attributes to different animals, especially waterfowls such as goose and duck where it eat Azolla very greedily. He explained that, for example, in order to prepare a good feed mixture for ducks, he mixes 4 kg of Azolla and a quarter of a kg of rice extract, fine salt (20-30 g), 20 cm^3 of methionine (a food supplement that ducks need, which is not It is located in Azolla. Dr. Hassan stressed that the results were excellent and better than the case of using traditional feed alone.

In this section the researcher Interviewed some Palestinian farmers and researchers in the West Bank and Gaza Strip that used Azolla as a green fodder to feed their animals.

The first interview: a group of farmers from Gaza Strip who were contacted remotely by phone. The most important questions asked to each of them was:

1. What was the reason that prompted the you to grow this plant?
2. Did you noticed any noticeable positive changes in the growth rate of the animal, the rate of production or the quality of production?
3. What is the percentage of Azolla that is used to feed to animals?
4. What is the most prominent difficulties that farmers faced with the Azolla plant?

In general, all the farmers in Gaza Strip answered the first question by saying it is because of the Israeli siege on Gaza Strip and the high prices of animal feed, farmers resorted to searching for alternative ways to provide fodder. One of the most important of these methods was the cultivation of Azolla and its incorporation in studied proportions into the traditional feed as part of the main meals for the animal.

Below is a breakdown of the rest of the questions, as the answers differed slightly from one farmer to another, each according to his experience.

With regard to the most prominent difficulties and challenges facing farmers in Azolla cultivation. The answers of most farmers were summarized in two main things, the first challenge for most of them was lack of information on the correct organic fertilizers suitable for Azolla, except for chemical fertilization. The second challenge was that most of them do not have a clear conception of the foundations of the fodder ration industry, which includes the Azolla with its accompanying additives based on sound scientific foundations.

Second interview: **Ibrahim Al-Zari'i** (Deir Al-Balah),

He is considered one of the first farmers of Azolla plant in Palestine in general and Gaza Strip in particular, he assured us that he used Azolla in raising ducks and municipal chickens. He added that he started his experiments on the plant at the end of 2016 after smuggling the plant from the Arab Republic of Egypt. He told us that he had tried cultivating two types of Azolla plant, namely *Azolla pinata* and *Azolla nilotica* (better in winter) and said that he started experimenting with the cultivation of the second type recently in early 2022. He added that *A.nilotica* bears lower temperatures better than the *A. pinata*. Moreover, he told us that he had introduced Azolla into the fodder at a rate of (70%). Before serving it to the birds, he had filtered it from the water for at least 12 hours (from evening hours until morning hours), And he used a special mixing machine to made his recipes.

Third interview: Khaled Al-Zuraei

He is another farmer from Gaza Strip started cultivating Azolla after he saw it with his friend Ibrahim Al-Zari'i. At first, he did an agricultural experiment on an area of 55 m^2, and when he succeeded, he decided to expand the area to become 200 m^2. Now, he is using the output to feed his five cows and a calf. He told us that his cows eat the plant greedily, cows significantly increased their milk production, he didn't provide specific percentages.

Fourth interview: Najah Abu Nada

She is a farmer, and an agricultural engineer who decided to go through the pioneering experiment in the field of non-traditional fodder in order to develop solutions to a problem she faced during her fish farming project. She told us that she wanted Azolla and transferred it from the Egypt, but all attempts failed and in the end she was able to introduce the plant to the Gaza Strip through a postal parcel from Europe to our occupied lands and then to the Gaza Strip.

She has told us that she used Azolla in fish feed at a high rate of more than 80%, and a decrease in the rate of fish growth was observed compared to the traditional feed for it. After conducting several experiments, she reduced the proportion of Azolla in fish feed by a rate ranging from 30-50 without any decrease with the growth rate or production with A noticeable improvement in the taste of meat. As for ducks, we have included Azolla in its feed mixture at a rate of 80-100% without any decrease in the rate of growth, reproduction or production with a noticeable improvement in the quality of meat. With regard to laying hens and broilers, she had introduced Azolla into the feed at a rate of 30% without causing any decrease in the growth rate of the bird or its reproduction rate. On the contrary, a 10-15% increase in the rate of egg production was observed in laying hens of various types. In addition to an improvement in the taste of eggs with a slight difference in the color of the yolk that is orange-yellow. There was a noticeable improvement in the quality, taste and tenderness of the meat of broiler chickens.

When we asked about the percentages that she used in raising sheep and cows, she assured us that after conducting several experiments, she found that the introduction of Azolla at 30% in the feed mixture for both sheep and cows did not show any decrease in the growth rate of the animal. And on the contrary, the animal's health improved and milk production was increased by 15-20%. Also, there was changes in the quality of meat in terms of taste and tenderness .

Fifth interview: Wael Muslim

A Palestinian farmer from Gaza Strip, he told us that he uses Azolla in raising fish, ducks, chickens and calves. He told us that he used Azolla in a large percentage exceeding 70%, but this did not prove its effectiveness and we used to try it randomly. Now, he is adding Azolla into the composition of the feed at a rate of 30% after filtering it from water for 4 hours. He stressed that the animal's immunity increases, egg production, taste and color increase, milk production increases, meat tastes better, its texture is better, and it is soft.

Sixth interview: Engineer Firas Qallawa (Jenin)

He is an agricultural engineer who started working on Azolla in late 2019 in order to complete a university research to obtain a bachelor's degree in agricultural engineering. In one of the villages of Jenin city, he conducted an agricultural experiment during which he used Azolla in the feed of broiler chickens at a rate of 40-50%. He confirmed that he was able to save about half the price of the feed and noticed a clear improvement in the taste of chicken meat that had feed on Azolla. The production of One dunum of Azolla is sufficient to feed approximately 1500 birds.

Seventh interview: Hani Nassar (Nablus)

He is an animal breeder from Nablus confirmed to us that in 2021 he conducted an experiment to introduce Azolla into the feed of municipal, rahmi and Chinese chickens (500 chickens), they are fed 100% traditional fodder.

The summary of all these interviews was as follows:

A) Azolla can be used in animal feed, such as municipal chickens, ducks, sheeps, cows, and calves.

B) The used percentage of Azolla varies according to the type of animal and there were differences in the percentages that each farmer used successfully. But most of them used Azolla for ducks, fish, local chickens at ratios of 70% or more. For sheep and cows, Azolla was introduced in percentages from 30-50%, the same was with broiler chickens

C) Before using Azolla in fodder, farmers filter Azolla from water for an average of 12-24 hours, in order to avoid infecting animals with diarrhea.

D) All of the farmers noticed tangible changes in their animals represented by regular and healthy natural growth, sometimes at rates greater than normal. They also noticed an improvement in the general health of their animals and less use of various medicines. They also noticed an increase in the rate of production of eggs and milk, and they also recorded an excellent improvement in the texture of meat And the taste in particular.

Chapter 4

Conclusions and Recommendations

4.1 Conclusions

The summary of the results that we reached through the set of experiments that we conducted on the Azolla plant confirms beyond any doubt the possibility of cultivating this wondrous plant in the environment and climate of the State of Palestine in all its regions (north, south, east and west). Locally, without any use of chemical fertilizers, using only agricultural soil and dried and decomposed organic fertilizer, where the plant doubled once every 4 days, and the mass of one square meter of Azolla plant after drying for 8 hours in the shade equals 800 – 1000 g, and thus the rate of production of a dunum in the results We got the equivalent of 6 tons per month. The results also showed that the water of fish farming ponds is considered an excellent fertilizer if it is used to grow Azolla on it.

The results showed the possibility of cultivating Azolla plant on secondary treated wastewater, but it can't be used as animal feeding since it can accumulate dangerous and heavy metals and so this can be transferred to humans if they eat these animals. Instead of that Azolla can be used for the production of biofuel. For the purpose of stirring and fertilizing only and in measured quantities (depends on the type of fish and its age or size), as the results of the experiments showed that vermicompost is the best ever in fertilizing the Azolla plant and can be used without agricultural soil. The experiments also concluded that the necessary heat is provided to achieve the best growth For the Azolla plant, this can usually be provided by growing it inside greenhouses.

It can be concluded that there is a need to provide natural or artificial shade to achieve the best growth rate for the Azolla plant, and this is usually done using a 50% shade net. The results of the experiments also showed the possibility of cultivating Azolla at different heights of water, but it is preferable to increase the depth of water during the summer to exceed 20 cm in order to control the water temperature in the basin more, and the rate of water consumption is relatively low and almost similar to the rate of water consumption in the cucumber plant, for example. Moreover, it was found that there is a need to cover Azolla ponds that are grown outside the greenhouse during frost days and cold days and nights.

The experiments also showed the importance of moving the water and soil of the Azolla ponds, especially in the summer. Results from local farmers show that the animals relish eating the Azolla plant and even like to eat it greedily. Through documenting some local experiences of cultivating the Azolla plant in different regions of Palestine, it was found that the Azolla plant could be introduced into animal feed in varying proportions, according to the type of animal. They also mentioned that the production of milk and eggs of these animals increased by 20%-30%. They also mentioned that the taste of the meat of their animals and the taste of eggs and milk that they produce is much better than before. They mentioned that they were able to reduce the cost of production by almost half after using the Azolla plant, and their use of preventive medicines was greatly reduced. Therefore, it is advised to cultivate Azolla plant in all regions of Palestine.

4.2 Recommendations

Based on the results of the experiments that I conducted on the Azolla plant, I came up with a set of recommendations, the most important of which are:

❖ **For governmental organizations and departments**

- Its recommended for the Palestinian Ministry of Agriculture to hold intensive training and workshops to educate farmers about the importance of this plant, methods of its cultivation, methods of using it in nutrition and the prospects for its environmental and agricultural uses.
- I advise the Palestinian Ministry of Agriculture to work on developing smart technological systems in order to facilitate the cultivation of the Azolla plant on wide areas of land with minimal effort, time and financial expenditure.
- It's advisable for the Palestinian Environmental Quality Authority to encourage farmers to cultivate Azolla on secondary treated wastewater in order to contribute to its purification (used as a bio-filter) and then the biomass product is used for the production of liquid biofuel and biogas.
- I advise the political decision-makers in the Palestinian Authority to consider the Azolla plant an issue of food security and an issue of agricultural independence, and

to deal with this plant as part of the battle for agricultural liberation from the occupation.

❖ For Researchers

- I advise all Palestinian researchers interested in the Azolla plant to work on cross-breeding types of Azolla that tolerate high salinity. In order to exploit this type of Azolla and later breed it on relatively salty water.
- I advise researchers to work on developing and cross-breeding species that have a higher growth rate and greater nutritional value, more than the existing ones.
- Its advisable for all researchers who are interested to intensify research and experiments on practical applications of the Azolla plant, especially with regard to increasing the proportion of Azolla in animal feed without losing any of its nutritional value.

❖ For Farmers

- It's advisable for all livestock breeders in the Palestinian territories who have the capabilities, to grow the wondrous Azolla plant, in order to include it in the animal feed ration in a certain proportion commensurate with the type and age of the animals, because of its ease in cultivation, and its high nutritional value, In addition to the many great health benefits for the animal, such as increasing the production of milk and eggs, improving the properties of meat and eggs and the general appearance, and increasing the conversion rate, and the great costs that this plant provides for them that were spent on feeding the animal.
- If the main purpose of investing in this plant is only to sell animal feed to other farmers, I do not advise investing in this plant, for several reasons, for example, that this plant is new to the Arab agricultural culture in general and the Palestinian in particular, as this plant entered the West Bank in the late 2019 only. Secondly, this plant loses its mass significantly after harvesting, and its mass decreases after drying, usually more than 90% of its mass when it is fresh compared to other plants such as wheat that can be stored and can preserve its mass. But if the purpose of its cultivation is to feed the animals of the farm owned by the farmer, in this case I would strongly advise.

- Its recommend to use the water of fish breeding ponds for fertilizing Azolla ponds due to its rich content in ammonia and the nutrients necessary for the growth of this plant, As well as building an integrated sustainable farm, one of its components being fish breeding basins and adjacent to the Azolla planting basins, which it was fertilized with the water of the fish basins, and the usage of a large part of the Azolla plant to feed the fish.
- Its recommend to use vermicompost as an organic fertilizer for fertilizing Azolla basins, because this fertilizer has a strong effect on the rate of plant growth, and because it is an unpolluted organic fertilizer that can be produced in simple ways locally.
- Its recommend to grow the Azolla plant inside a greenhouse to provide the heat necessary for the growth of this plant at a large rate, because the origin of this plant is tropical and therefore it combines its love for heat and humidity at the same time.
- Its advisable to cover the Azolla plant with a transparent plastic cover during the cold days and months , in case there are no possibilities to build a greenhouse, to protect the plant from frost at night. It is also recommended to cover the basins planted with Azolla during the night hours only, if they are planted in the desert (due to the extreme cold in the desert climate during the night hours) or during the nights when there is frost in order to preserve the plant.
- Its advisable to provide suitable shade through the use of shading nets that allow 50% light penetration.
- Its recommend to increase the depth and height of the water in Azolla basins during the hot days and months, in order to control the temperature of the water in the basin and prevent it from rising significantly, because the high temperature of the basin reduces the efficiency of the roots' absorption of nutrients and thus the rate of decrease Growth and reproduction, and vice versa during cold winter days.
- Based on the interviews I conducted with many farmers who grow using soil and animal manure only, its recommended that the thickness of the soil placed in the basin should be no less than 10 cm, because of its importance in regulating the temperature of the basins in summer and winter.
- Based on the observations that we made when planting Azolla in the winter season, Its recommend to reduce the area of the basin that is harvested during the winter season, in order to maintain the thickness of the Azolla layer that covers the surface of

the basin and forms a thermal insulator that maintains the temperature of the basin water as much as possible and thus maintains the rate of absorption of elements Thus, the rate of plant growth and reproduction in the aquarium.

- Its advise farmers who use agricultural soil for fertilization in addition to organic fertilizer to move the agricultural basins on a permanent and daily basis, at least twice a day, because moving the basin water plays an important role in regulating the temperature of the basin water and ensuring the arrival of The largest amount of elements for the roots of the Azolla plant. Thus, the rate of growth and reproduction increases, especially in the summer .
- Its advisable for farmers to make the Azolla ponds self-fertilize by raising a certain number of fish inside these ponds so that the farmer ensures that these fish move the water and soil of the pond, in addition to that, the fish fertilize the plant from Through its feces rich in nutrients, which it throws into the water. At the same time, there is no need to feed the fish because the Azolla is its food, but in this system, the density of the fish is taken into account, so that it does not exceed a certain amount and does not fall below a certain limit, because increasing the number of fish means burning the Azolla because of the increase in the concentration of its chapters and also means that the fish will devour all Azolla, meaning that the thickness of the fish is too low, that the fertilization is not enough.
- Its recommend to sterilize the organic fertilizer (especially if it is a fresh, not fermented or dry green fertilizer) before adding it to the Azolla basin for fertilizing, by mixing the solution or fertilizer soaked with copper sulfate or any sterile compound with a percentage of Sufficient is usually 5 g/L. This is in order to ensure that molds, bacteria, fungi and various algae do not grow in the Azolla basin.
- Its also recommend the production of vermicompost through the use of the Azolla plant.
- Its recommend using Azolla plant in anaerobic reactors in order to increase the percentage of biogas that is produced.
- I recommend the use of this plant in the production of organic fertilizer, which is characterized by being free of any odors (especially rose nurseries).
- I advise the Palestinian families to exploit the rooftops of buildings in order to grow this plant, which can be used as food for humans in times of hardship. At the

same time, the Azolla basins on the roofs of buildings act as a thermal insulator for the roofs and keep the house warm in summer and winter .

- I recommend planting Azolla in palm and olive farms that are characterized by flat ground, in order to make greater use of the space and at the same time provide trees with suitable shade for the growth of Azolla .
- I recommend planting this plant in lands characterized by saline or polluted soils that are not suitable for cultivation, especially arid desert lands. This is in order to provide agricultural land for the cultivation of other types of food plants that humans need.

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Appendices:

Appendix Table 1: Comparison of the growth rate of Azolla fertilized using vermicompost and Azolla fertilized with chemical fertilizer.

Treatments	basins number	Azolla initial Quantity (g)	after 4 days	8 days	12 days
Fourth group, fertilized with vermicompost	1	50	113	255	574
	2	50	118	267	600
	3	50	115	259	583
Fifth group, fertilized with nutritional solutions	1	50	104	229	503
	2	50	110	242	532
	3	50	106	233	512

Appendix Table 2: The growth rate of Azolla compared to the growth rate of the water lentil plant.

plant type	planted quantity (g)	Biomass 2/1/2021	12/1/2021
Azolla	50	122	305
water lentils	50	137	382

Appendix Table 3: The growth rate of Azolla compared to the growth rate of the water lentil.

		Biomass (g) every 4 days					
Plant type	basin number	03/12/2020	07/12/2020	11/12/2020	15/12/2020	19/12/2020	23/12/2020
Azolla	1	210	223	217	208	219	216
	2	215	227	216	211	217	221
	3	190	212	2 22	202	224	214
water lentil	1	252	240	251	242	265	257
	2	228	254	253	240	269	261
	3	258	260	244	249	270	247

Appendix Table 4: Average growth rate of Azolla compared to the average growth rate of the water lentil.

	Average biomass (g) every 4 days					
plant type	03/12/2020	07/12/2020	11/12/2020	15/12/2020	19/12/2020	23/12/2020
Azolla	205	220.6	218.3	207	220	217
water lentils	246	251.3	249.3	243.6	268	255

Appendix Table 5: Effect of water quality on the rate of reproduction of Azolla plants.

Treatments	basin number	20/1/2021	25/1/2021	30/1/2021	04/02/2021	09/02/2021
Wastewater treatment secondary treatment G1	1	221	219	213	209	203
	2	218	217	223	218	206
	3	216	212	214	218	201
fish ponds water G2	1	235	239	233	240	229
	2	238	234	235	234	221
	3	232	236	237	233	224
Ordinary drinking water from the well, with only dirt G3	1	191	188	198	192	183
	2	186	195	197	191	189
	3	199	203	189	205	186

Appendix Table 6: Average Azolla biomass after 5 days of culturing on sources of water.

Treatments	basin number	Growth rate in 5 days
Wastewater treatment secondary treatment G1	1	213
	2	216.4
	3	212.2
fish ponds water G2	1	235.2
	2	232.4
	3	232.4
Ordinary drinking water from the well, with only dirt G3	1	190.4
	2	191.6
	3	196.4

Appendix Table 7: The effect of moving soil and water in the ponds on the rate of reproduction of Azolla during winter.

Treatments	basin number	20/12/2020	29/12/2020	8/1/2021	6/1/2021
Group A (without moving)	1	50	103	211	430
	2	50	104	212	432
	3	50	102	208	426
Group B (with moving)	1	50	112	227	476
	2	50	114	239	497
	3	50	119	245	507

Appendix Table 8: The effect of moving soil and water in the ponds on the rate of reproduction of Azolla during summer.

Treatments	basin number	1/7/2021	6/7/2021	11/7/2021	16/7/2021
Group (A) without moving	1	50	89	169	321
	2	50	98	186	353
	3	50	95	181	344
Group B by moving	1	50	113	248	546
	2	50	111	244	537
	3	50	108	237	522

Appendix Table 9: The difference in the growth rate of Azolla grown inside the greenhouse compared to the growth rate of Azolla grown outside.

Treatments	basin number	20/3/2021	25/3/2021	30/3/2021	5/4/2021
Inside the greenhouse	1	50	110	232	480
	2	50	119	255	527
	3	50	114	237	485
outside the greenhouse	1	50	83	152	276
	2	50	79	149	264
	3	50	88	159	289

Appendix Table 10: The growth rate of the Azolla plant inside the greenhouse during the summer compared to its growth rate during the winter.

		Average biomass (g)				
planting time	basin number	20/1/21	25/1/21	30/1/21	05/02/2021	10/2/201
winter season -2021/1/20) (2021/2/20	1	100	152	229	341	528
	2	100	144	216	324	493
	3	100	161	249	374	576
summer season -2021/6/10) (2021/7/10		10/06/2021	15/6/21	20/6/21	25/6/21	30/6/21
	1	100	223	490	989	2007
	2	100	229	503	1016	2062
	3	100	219	481	971	1971

Appendix Table 11: The effect of covering Azolla ponds with a transparent plastic cover during cold days and frost nights on their safety and reproduction rate.

		Average biomass (g)			
Treatments	basin number	planted quantity	After a week	Two weeks later	3 weeks later
Group A (basins covered)	1	50	105	221	464
	2	50	108	227	477
	3	50	104	219	460
group B (basins not covered)	1	50	75	113	169
	2	50	71	107	161
	3	50	73	109	164

Appendix Table 12: The effect of sterilization on the safety and reproduction rate of Azolla ponds.

		Azolla biomass (g)		
Treatments	basin number	20/3/2021	27/3/2021	4/4/2021
The first group (non-sterile organic fertilizer)	1	50	76	99
	2	50	68	87
	3	50	82	106
	4	50	74	96
The second group (sterile organic fertilizer)	1	50	109	241
	2	50	103	227
	3	50	107	235
	4	50	102	224

Appendix Table 13: Studying the effect of different types of fertilizers on the growth rate of Azolla plants.

	Azolla biomass (g)			
Treatments	15/7/2021	20/7/2021	25/7/2021	30/7/2021
First group Organic fertilization with cow dung	113	254	571	1284
	111	249	560	1260
	115	258	579	1302
Second group, Organic fertilization with sheep manure	107	230	494	1062
	104	224	481	1034
	106	228	490	1053
Third group, Organic fertilization with pigeon and chicken manure	110	242	532	1170
	109	239	526	1157
	109	243	535	1176
Fourth group, Organic fertilization of fish pond water	115	264	607	1396
	117	269	618	1421
	114	262	603	1386
Fifth group, Organic fertilization with vermicompost	125	312	781	1952
	123	308	772	1933
	126	315	788	1969
Sixth group, Chemical fertilization with nutrient solutions	120	288	691	1658
	121	290	696	1671
	118	283	679	1629
Seventh group, Water only, without any fertilizers.	55	51	48	43
	58	53	50	46
	53	49	45	42

Appendix Table 14: Cultivation of Azolla at different heights of water and its effect on the rate of reproduction .

Treatments	Basin number	Planted quantity (g)	Biomass after 15 days	Average biomass (g)
Group 1 20 cm depth	1	50	569	568.3
	2	50	564	
	3	50	572	
Group 2 depth 15 cm	1	50	535	532
	2	50	528	
	3	50	533	
Group 3 10 cm depth	1	50	456	456.6
	2	50	463	
	3	50	451	
Group 4 5 cm depth	1	50	385	378.6
	2	50	372	
	2	50	379	