EFFECTS OF NITROGEN CONCENTRATION AND SHOOT HARVESTING FREQUENCY ON THE REGROWTH OF SHOOT AND DEGENERATION OF ROOT OF WATER HYACINTH (*Eichhornia crassipes*) GROWN ON POT AT HAWASSA, ETHIOPIA

M.Sc. THESIS

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HAWASSA UNIVERSITY

COLLEGE OF AGRICULTU

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THESIS SUBMITTED TO HAWASSA UNIVERSITY DEPARTMENT/SCHOOL OF PLANT AND HORTICULTURAL SCIENCES, COLLEGE/INSTITUTE OF AGRICULTURE, SCHOOL OF GRADUATE STUDIES, HAWASSA, ETHIOPIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN PLANT SCIENCES AND HORTICULTURE (SPECIALIZATION: HORTICULTURE)

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COLLEGE OF AGRICULTURE
SCHOOL OF PLANT AND HORTICULTURAL SCIENCES
ADVISORS 'APPROVAL SHEET
(Submission Sheet – 1)

This is to certify that the thesis entitled " Effects of Nitrogen Fertilizer Rates and Shoot Harvesting Frequency on the Regrowth of Shoot and Degeneration of Root of Water Hyacinth (*Eichhornia crassipes*) grown on pot" at Hawassa, Ethiopia, is submitted in partial fulfillment of the requirements for the degree of **Masters of Sciences** with a specialization in **Horticulture** Graduate Program of the School of **Plant and Horticultural Sciences**, College of Agriculture, and is a record of original research carried out by **Yedidya Biratu**, under our supervision, and no part of the thesis has been submitted for any other degree or diploma. Therefore, I recommend that the student has fulfilled the requirements and can, therefore, hereby submit the thesis to the department.

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(Submission Sheet – 2)

We, the undersigned, members of the board of examiners of the final open defense by Yedidya Biratu, have read and evaluated her thesis entitled "Effects of Nitrogen Fertilizer Rates and Shoot Harvesting Frequency on the Regrowth of Shoot and Degeneration of Root of Water Hyacinth (Eichhornia crassipes) grown on pot" at Hawassa, Ethiopia, and examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree of Master of Science in Plant and Horticultural Sciences (Horticulture program).

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Statement of the Author

I, Yedidya Biratu, declare that this thesis is my real work and the original report of my research that has not been submitted to any other institution in whole or in part for any other degree, diploma, or certificate. This thesis has been submitted in partial fulfillment of the requirements for a M.Sc. degree in horticulture at Hawassa University.

Yedidya Biratu	______________________	____________________
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LIST OF ABBREVATIONS AND ACRONYMS

ANOVA	Analysis of Variance
BSc	Bachelor of Science
E	Transpiration Rate
EEA	European Economic area
EC	Electric Conductivity
EELPA	Ethiopian Electric Light and Power Authority
FW	Fresh Weight
CRD	Complete Random Design
KNO_3	Potassium Nitrate
LSD	Least Significance Difference
MSc	Masters of Science
N	Nitrogen
NO_3^-	Nitrate
$(NH4)_2CO_3$	Ammonium Carbonate
PT	Palin Test
RH	Relative Humidity
RLWC	Relative Leaf Water Content
SAS	Statistical Analysis Software
TDS	Total Dissolved Solid
TSC	Total Structural Carbohydrates
TNC	Total Non-structural Carbohydrates
USA	United States of America
VPD	Vapour Pressure Deficit
WUE	Water Use Efficiency

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ABSTRACT

Water hyacinth (Eichhornia crassipes) is a perennial, herbaceous, free-floating, flowering, and invasive aquatic plant of the genus Eichhornia in the Pickerelwe family (Pontederiaceae). Recently, water hyacinth has been considered an invasive aquatic plant in Ethiopia. The main control methods of water hyacinth in Ethiopia are manual harvesting or weed removal by hand. The problem related to manual harvesting: there is no recommended harvesting schedule concerning the regeneration potential of the weed, and there is no integrated management practice. Therefore, a pot experiment was conducted to investigate the effect of nitrogen fertilizer and harvesting frequency on the regrowth of shoot and root degeneration of water hyacinth, to evaluate the response of water hyacinth to different rates of nitrogen fertilizer application, and to evaluate and determine the optimum harvesting frequency that degenerates the regrowth of shoot in water hyacinth under shade house conditions. The treatments comprised a factorial combination of six nitrogen fertilizer levels (0, 20, 40, 60, 80, and 100 mg L^{-1}) and four harvesting levels (unharvested, harvested once, harvested twice, and harvested thrice) with three replications. A total of 24 treatment combinations were formed. The pot experiment will be set out as a complete randomized design (CRD). The results revealed that all growth parameters were significantly influenced by the mean factor as well as the interaction. When it comes to root growth, the treatments with 0 and 20 mg L^{-1} nitrogen treated without harvest had the greatest root length, root diameter, and root number. All gas exchange parameters are also influenced by the interaction effect of the main factor, except leaf relative water potential and stomatal conductance, which are both only affected by the main effect. Chlorophyll a and b and total chlorophyll are only influenced by nitrogen fertilizer. Water hyacinth exposed to frequent harvesting significantly reduced all tested parameters except stolen production. The present result, therefore, indicates that 60 mg/L^{-1} nitrogen fertilizer is the best for the growth of water hyacinth and that harvesting frequency decreases the regrowth of shoots and degeneration of roots. Therefore, we recommend management practices that reduce water body's pollution and frequency of harvesting to degenerate the root of water hyacinth and its impact on the water bodies

Key words: - plant growth, regrowth potential, Stolon production, shoot harvesting and Water hyacinth

1. Introduction

Water hyacinth (*Eichhornia crassipes*) is a perennial, herbaceous, free-floating, and flowering invasive aquatic plant of the genus *Eichhornia* in the pickerel weed family (*Pontederiaceae*), consisting of about five species (Gopal, 1987; Patil *et al., 2012*). Some water hyacinth species float in shallow water, while others grow on muddy stream banks and lakeshores. It was first observed in the Amazon basin, South America. It has spread mainly to the tropics and subtropics since the 1800s (Mujere, 2016). The species were identified by a German naturalist, C. Von Martius (Tellez *et al., 2008*). It gained attention as an ornamental plant because of its attractive purple flower. Water hyacinth (*Eichhornia Crassipes*) is probably the most invasive and prolific plant species in the world's rivers, lakes, and canals (Nyananyo et al., 2007).

Water hyacinths have slender rootstocks, feathery roots, rosettes of stalked leaves, and few too many flowers arranged in spikes or clusters in the leaf axils (Gloria, 2020). Its leaves are spongy and inflated, and the upper lobes of the purple flowers have blue and yellow markings. It reproduces quickly and often clogs slow-flowing streams. It is used as an ornament in outdoor pools and aquariums. Owing to its extremely fast growth, it soon became the major floating waterweed in tropical and subtropical regions worldwide (Groote *et al., 2003*).

In a multi-criterion analysis, the major factors controlling the growth of water hyacinth in water bodies could be categorized as climatic conditions (temperature, humidity, and sunlight shading), water body conditions (salinity of the water, disturbances like flooding, current and wave directions), eutrophication (nitrates, phosphates, and sulfates in dissolved form), pH, and the reproduction system (sexual and asexual) of the invasive weed (Dersseh *et al., 2019).* Quiescent water, shallow water depth (<6 m), bed surface covered with deposited sediment rich in organic matter, availability of vital elements such as nitrogen and phosphorous nutrients, and agreeable temperature conditions are the most favorable conditions (Dersseh et al., 2019).

According to Gaikwad and Gavande (2017), water hyacinth can reproduce both sexually (by seeds) and asexually by vegetative means: (budding and stolen production). Both reproduction systems have a large production potential within a very short period of time, but vegetative reproduction is the most prolific (Lugard and Ogukwe, 2007). The rapid increase and spread of the plant into new areas are due particularly to its vegetative reproduction, which allows a single plant to develop very rapidly into a significant infestation. Moving easily with water currents, winds, or other accidental means, such as fishing nets and boats, the plant invaded rivers, canals, ponds, lakes, dams, and other freshwater bodies (Tellez et al., 2008; Tewabe et al., 2017).

In Ethiopia, water hyacinth plants were first introduced and observed in the rift valley lake region in the year 1956 at Koka Dam and the Awash River (Gedefaw and Gonder, 2018). Ethiopia's Electric Light and Power Authority (EELPA) reported the weed as a problem disrupting its hydroelectric operation at three stations located along the Awash River, where it emerges from Koka Lake and then distributes to other bodies of water over time (Firehun et al., 2014). As we know, water hyacinth is one of the most invaded weeds in most Ethiopian lakes and rivers. The exact cause and source of the water hyacinth infestation in Ethiopia's lake are not clearly known yet, but they are probably favored by factors such as sedimentation, extensive fertilizer application in the agricultural parts of the catchment, and pollutants (nutrients) from the environment around the lake (Dersseh et al., 2019). Since then, it has emerged as one of the ten most ecologically risky aquatic flowers in the country. It has inflicted an adverse impact on the social, economic, and livelihood conditions of the people and development projects in the Rift Valley Lakes Region (Dersseh et al., 2019; Enyew et al., 2020). The economic impact of water hyacinth in the invasion area of Ethiopian lakes and rivers has not yet been determined, with the exception of wonji-shoa sugar estate, which spent approximately US $100,000 on weed control from 2000 to 2013 (Firehun et al., 2014).

The productivity of water hyacinth are dependent on the available nutrient supply. Under most conditions, N is probably the major plant nutrient limiting productivity (Reddy and Tucker, 1983). Research indicates that N supply through urea produces maximum water hyacinth biomass production as compared to NO3-, (NH4)2CO3, or KNO3 (Shiralipour et al., 1981; Reddy and Tucker, 2016). Increasing concentrations of nitrogen and phosphorus result in increases in biomass accumulation, stolen (rament) production, shoot to root ratio, and plant height (Heard and winterton, 2000).

A water hyacinth is an aquatic perennial plant (Patil et al., 2012). Perennials and the ability to re-grow following grazing or harvesting are traits of agronomic value in perennial plants. It is likely that the ability of plants to store and remobilize carbon and nitrogen reserves in persistent organs plays an important role in determining their perennial lifestyle and/or their capacity for vigorous regrowth following grazing, cutting back or winter die-back (Vriet et al., 2014).

Water hyacinth control methods include reduction of water nutrients, use of physical barriers (manual and mechanical), and biological and chemical control (Karauach et al., 2022). For effective control, the plant must be removed faster than the rate of reproduction (Julien et al., 1996) by manual or machinery used harvesting. Intensified monitoring, mitigation, and management measures are needed to keep water hyacinth at unproblematic levels (UNEP, 2013). With due consideration of the problem related to water hyacinth invasion, several research and studies cited elsewhere in the world were conducted at different times and places. But there is no effective result or method for successfully reducing the expansion of the invasive water hyacinth in the water bodies (Karouach et al., 2022). In addition, the control of aquatic invasive plants like water hyacinth can not only stay in control of its biomass growth; the important goal should be to control the coverage area of invasive plants on the water (Gao et al., 2016).

Given the complexity of control options and the potential for climate change to assist the spread of water hyacinth, it is critical to develop comprehensive management strategies and action plans (Wainger et al., 2018). Studies showed that frequent harvesting decreases water hyacinth biomass yields, increases nutrient uptake and decreases detrital tissue accumulation and also thereby improving water quality. Frequent harvesting may also reduce the average plant age, regrowth potential and insect and disease problems of hyacinth plants (Reddy and D'Angelo, 1990).

In this study, we conducted a shade house experiment in which water hyacinth was grown at an experimentally manipulated level of nitrogen concentration and harvesting frequency. Our aims were to examine the relationship between water hyacinth regrowth potential and nutrient availability with harvesting frequency to test whether nutrient availability, especially nitrogen fertilizer, in the aquatic environment is a factor that limits the growth, regrowth, and spread of water hyacinth. Does harvesting frequency affect the regrowth potential of water hyacinth shoots and the degeneration of roots? Can we use frequent harvesting as one of the control methods? In Ethiopia, the main control method for water hyacinth expansion in the bodies was manual harvesting or weed removal by hand or by small-scale harvesters. The problem related to manual removal was that water hyacinth not only grows in the water bodies but also grows on well-drained land, shallow water or on muddy shores around the lake and the river. Since the weed is removed by hand, the root is often anchor in the mud shores, and it grows back quickly due to this recommended harvesting schedule needed and also its need integrated management practices because of its high expansion, regeneration, and reproduction capacities.

1.2 Objective

1.2.1 General objective

- To evaluate the effect of nitrogen fertilizer and harvesting frequency on the regrowth of shoot and root degeneration of water hyacinth.

1.2.2 Specific objective

- To evaluate the response of water hyacinth to different rates of nitrogen fertilizer application under partially controlled shade house conditions,

- To evaluate and determine the optimum harvesting frequency that degenerates the regrowth of shoots of water hyacinth

2. Literature Review

2.1. Water Hyacinth Taxonomy and Biology

A water hyacinth is a perennial, herbaceous, and aquatic plant species in the genus *Eichhornia* of the family *Pontederiaceae* (Patil et al., 2012). The leaves of water hyacinth are simple and basal, forming a rosette around the flower stalk, with a smooth, glossy, circular to kidney-shaped lamina and a spongy and swollen petiole(De thabrew, 2014) (Figure 1.1). The petiole of water hyacinth varies from long and slender to swollen or bulbous. The petiole helps the water hyacinth to float on the water surface because it contains air (Julien et al., 2001). The shape of the petiole influences the amount of air contained and the capacity of the water hyacinth to float (Julien et al., 2001; Firehun, 2017). The venation of the leaf is parallel, and the leaf margin is smooth. Stolons grow horizontally to produce daughter plants from terminal buds. The inflorescences are loose, spike-like clusters of tiny lavender blossoms borne on upright stalks. It bears between 8 and 15 violet and yellow flowers (Sharma and Aggarwal, 2020). Each flower has 6 petals; the lower 5 petals are a solid shade of lilac or lavender, but the uppermost petal has a bright yellow dot surrounded by a bluish "halo." The flowers can be self-fertilized. Each inflorescence produces 3000--4500 seeds (Xie and Yu, 2003; Sharma and Aggarwal, 2020).

The root morphology is highly plastic, fibrous, and has one main root with many laterals, forming a colossal root system. Because each lateral root has a root tip, water hyacinth may exploit nutrients in a low-nutrient water body (Tham, 2012). Lateral roots are generally longer and denser at low nitrogen levels than at high nitrogen levels (Xie and Yu, 2003). In shallow water, the roots may become attached to the bottom for several weeks when the water level drops. The root-shoot ratio varies inversely with the nutrient level, particularly with respect to nitrogen. Purple roots are characteristic of plants when nutrient levels are low in the water (Tham, 2012).

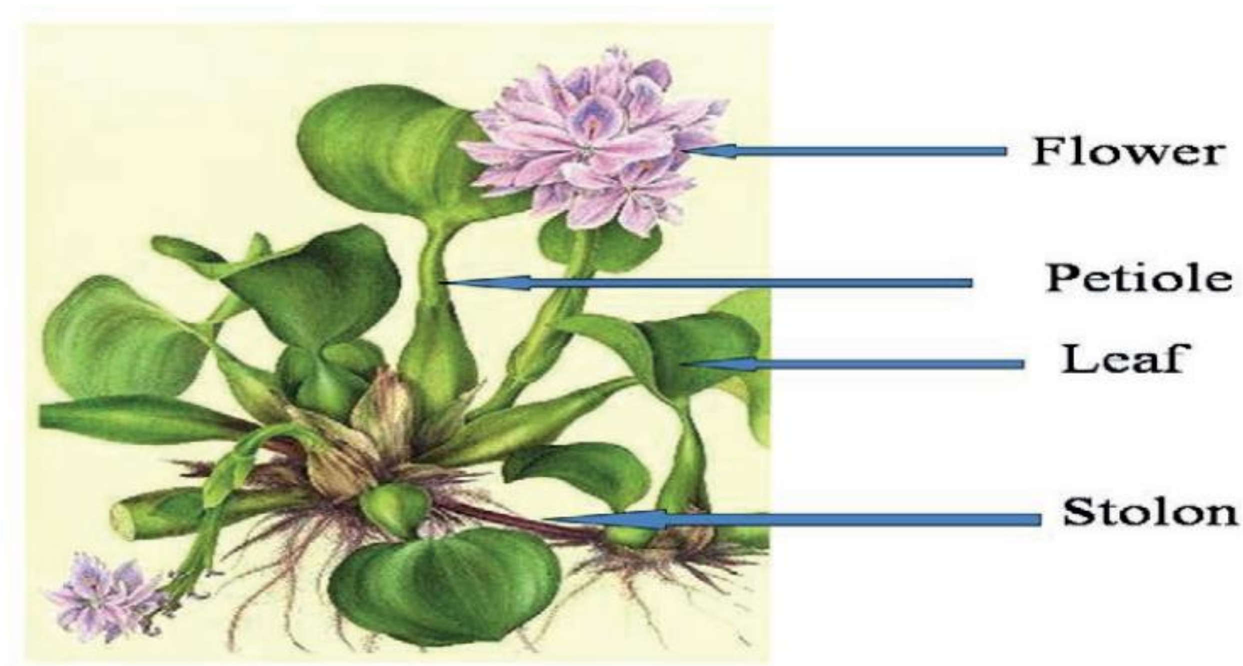

Figure 1.1 Morphology of water hyacinth

The seed can remain viable for up to 28 years in sediments (Gupta and yadav, 2020). Water hyacinth seeds germinate in backwater areas, in ditches and channels, and in other moist habitats (Ndimele et al., 2011). Within 40 days after germination the seedlings develop into plants with their own leaves and root systems. Vegetative propagation is a common form of propagation and is largely responsible for the rapid spread of water hyacinth in new areas (Ingwani et al., 2010). By way of runners or stolons, the daughter plant develops its own roots. Once the rament has developed its own roots, the stolons either decay or break and separate from the mother plant (Gupta and Yadav, 2020).

Characteristics of water hyacinth

Water hyacinth has the following systematic, morphological, developmental, biological, and ecological characteristics: A perennial plant that grows to a height of 1 m and has an average size of 40 cm and it's have the capacity to regrowth after cutting, harvesting and wooding (Magar et al., 2017). They have a rapid vegetative growth and multiplication rate; produce seeds that remain viable for very long periods, up to 28 years (Gupta and yadav, 2020). They have a fairly broad ecological amplitude (Khalil et al., 2009). Stems and leaves contain air-filled parenchyma tissue, which gives the plant its considerable buoyancy and helps it float in the water (Julien et al., 2001). Asexual vegetative reproduction allows it to double its population in 15–18 days (Gupta and Yadav, 2020). Each mother plant produces four daughter plants, which are capable of reproduction after 2 weeks. Growth is greatly accelerated in nutrient-rich, eutrophic water bodies with high nitrate and phosphate concentrations. (Gupta and Yadav, 2020). It has heavy metal and nutrient absorption capacities (Ingole and Bhole, 2003; Sanmuga and Senthamil, 2014).

2.2 Water Hyacinth Occurrence and Distribution

Water hyacinth has spread to all continents, except Antarctica (Awasthi et al., 2013). It was introduced from the Amazon basin, South America (Mujere, 2016). Since the end of the nineteenth century, the plant has been taken from its origin to all parts of the world as an ornamental and botanical garden plant because of its attractive purple flower (Tellez et al., 2008). But due to limited knowledge about the water hyacinth's characteristics, it became out of control and has become the most dangerous weed in water bodies throughout the world (Julien, 2000).

In the USA, water hyacinth was first introduced in Louisiana in 1884 and afterwards in Florida in 1890 (Pan et al., 2012). Because of its striking flowers, it was deliberately introduced into botanic gardens in many other countries, from which it inevitably spread as a weed (CABI, 2015; Gedefaw and Gonder, 2018).

In Europe, water hyacinth has been established in the Azores (France) and in Corsica (Italy), and casual records are known from Belgium, the Czech Republic, Hungary, the Netherlands, and Romania. Water hyacinth is also a threat in Spain and Portugal (Keawmanee, 2015).

In Asia, water hyacinth is widespread in freshwater wetlands of the Mekong Delta, especially in standing water (MWBP/RSCP, 2006). Yigermal et al. (2020) reported that water hyacinth has been detected in the Sundarbans mangrove forest of Bangladesh and has caused heavy siltation in the wetlands of the Kaziranga National Park, India.

In Africa, the earliest reports on the water hyacinth were from Egypt in the late 1800s. Today, however, it is present in the freshwater bodies of practically all countries in the region (Navarro and Phiri, 2000).

In Ethiopia, the first reports on the water hyacinth were from Koka dam and the Awash River in 1956 (Degaga, 2018). Ethiopia's Electric Light and Power Authority (EELPA) reported the weed as a problem disrupting its hydroelectric operation at three stations located along the Awash River, where it emerges from Koka Lake and then distributes to other bodies of water over time (Enyew et al., 2020).

2.3 Water Hyacinth Ecology and Invasion in Ethiopia

Climate change has profound effects on the distribution of water hyacinth. The favorable habitat or environment for the growth of water hyacinth ranges from tropical to subtropical or warm temperate to rainforest zones (Gakiwad and Gavand, 2017). The rapid reproduction rate of water hyacinth is because of two major conditions: climatic and water body conditions Temperature, sunlight shading, salinity, disturbance, eutrophication, pH, and reproduction systems are the main determinant factors for the optimum growth of water hyacinth (Dersseh et al., 2019). Quiescent water, shallow water depth (<6 m), a bed surface covered with deposited sediment rich in organic matter, and the availability of vital elements such as nitrogen and phosphorous in nutrients are the most favorable conditions (Dersseh et al., 2019). The optimal salinity condition for water hyacinth is <2% Flooding, waves, and currents are the main disturbances to the stability of the weed because high flooding, waves, and currents can flush out the weed downstream (Minychl et al., 2019). The favorite nutrients are nitrates, phosphates, and sulfates in dissolved form. The source of these nutrients could be eroded nutrients from agricultural fields, industrial zones, and residential areas. The plants can tolerate pH values from 4 to 10, and also withstand frost (Dersseh et al., 2019).

In Ethiopia, water hyacinth has been reported to invade two major areas of the country: the Nile basin and the awash basin, extending down to the rift-valley region (Firehun et al., 2014). Water hyacinth was officially first reported about 66 years ago in Koka Lake and the Awash River in 1956 **(**Firehun, 2017). It is known as "Emboch" in Amharic or "Bocee" in Afan Oromo (Teshome and Amente, 2021). Water hyacinth was less recognized in water bodies from late 1956 to late 2011, but the infestation rate has now been manifested on a large scale in many water bodies throughout the country (Firehun et al., 2014). The introduction and rapid spread of this weed in the Awash River Basin (Koka Lake, Koka Dam), Abbay River Basin (Lake Tana, Blue Nile), Baro-Akobo River Basin (Sobate, Baro, Gillo, and Pibor rivers) and Rift Valley Basins System (Lake Ellen, Lake Abaya, and Lake Elltoke) has created serious problems for the use of water as a resource (Dechassa and Abate, 2020).

The infestation area in Lake Tana was only 50.5 ha in 2012, and it's limited to one kebele. But as we know, the water hyacinth infestation increased in space and time and infested around 30 kebeles in nine districts (Takusa, West Dembia, East Dembia, Gonder Zuria, Libokemkem, Fogera, Dera, Bahir Dar Zuria) (Dechassa and Abate, 2020).

At Wonji–Shewa sugar estate, the invasion of water hyacinth started in 1956 due to flooding by overflow of the Awash River that crosses the Koka dam (Firehun et al., 2014). According to Firehun's et al., 2014 report, Lake Ellen was covered 100% with water hyacinth, while Aba Samuel Dam covered about 46%, Lake Koka covered 37%, and Abaya covered about <1%.

2.4. Water Hyacinth's Socioeconomic Impact in Ethiopia

The water hyacinth has a complex and multifaceted socio-economic impact in Ethiopia (Mengistu et al., 2017). The major impact of water hyacinth was interference with agriculture practices by blocking irrigation and drainage systems and by increasing the loss of water (Gedefaw and Gonder, 2018). It also affects the physical and chemical properties of the water by reducing temperature, pH, biological oxygen demand and nutrient level (Priya and selvan, 2017). High infestation of the weed restricts water flow in rivers and irrigation channels and causes structural bridge damage. Reduced irrigation flow can cause an indirect loss of field crops. According to Gebregiorgi (2017), he estimated the negative impact of the weed on crop production. The crop production of close to 70% of farmers was affected by water hyacinth in the Rift Valley of Ethiopia. And also, other researchers show that the loss of crop production varied across the country depending on the weed infestation rate. In severely affected crop fields, about 75 to 100% of crop production was lost (Enyew et al., 2020).

Due to vegetative reproduction, a vigorous growth rate and high organic load can lead to anoxic conditions that impact on the water column, such as fish and zooplankton (Mengistu et al., 2017). The plants also cover the entire surface of lakes and ponds, dramatically impacting water flow, blocking sunlight to native submerged plants, and preventing the transfer of oxygen from the air to the water surface (Nguyen et al., 2015; Gupta and yadav, 2020). And also the water hyacinth dies and sinks to the bottom in large numbers depletes the oxygen level and degrades water quality and quantity this result the dissolved oxygen levels to dangerous low concentration and often killing fish. (Bhattacharya et al., 2019). This all affects the fish reproduction, productivity, and the weed also competes for nutrients with other plants on which some fish feed (Never, 2015), as well as the fisherman's income due to increase in the cost of fish production. This related process goes from individual to community, from community to country, because in most rift valley cities' economies depend on fish markets (Enyew et al., 2020).

It is also a prime habitat for water-borne diseases and vectors, as the stagnant water caused by the weed's obstructing effect provides a breeding ground and habitat for several disease vectors, such as mosquitoes, flies, snails, and other organisms associated with human illnesses, such as malaria, schistosomiasis, encephalitis, filariasis, and cholera (CABI, 2015; Firehun, 2016). According to Senayit et al. (2004), an increased malaria incidence and lack of drinking water coincide during the peak infestation period, when the free-floating weed serves as an alternate host for snakes and crocodiles.

Various studies have been carried out to ascertain the relationship between aquatic plants and the rate of evapotranspiration compared with evaporation from an open-surfaced water body. The weed loses water rapidly through its broad leaves, which is about 3.5 times that of a free water surface (Dersseh et al., 2019). The flow of water in the Nile has been reduced by up to one tenth due to increased losses from evapotranspiration by water hyacinth in Lake Victoria (Ndimele et al., 2011).

The other socio-economic impact of water hyacinth in Ethiopia's economy was the effect of water hyacinth on many large hydropower schemes. The Koka hydropower dam was highly affected by the rapid reduction of water resources due to the water hyacinth problem and it affected the electric power production of the dam (Yigermal and Assefa, 2019).

2.5: Beneficial Uses of Water Hyacinth

Beyond its negative impact, water hyacinth has some amazing positive values, but only if it is done properly and in a way that we can control. One of the most amazing positive values is its bioremediation or phytoremediation properties. Phytoremediation is defined as the use of green plants to reduce organic and inorganic environmental pollutants (Singh and Balomajumder, 2021). Water hyacinth has all the phytoremediation characteristics. The characteristics are quick growth rate, high biomass yield, the capacity to uptake a large amount of pollutants, the capacity to transport pollutants aboveground, and a capacity to tolerate pollutants' toxicity (Singh and Balomajumder, 2021). Due to all these characteristics, it has been used for the removal or reduction of nutrient pollutants, heavy metals, organic compounds, and pathogens from water (Ingole and Bhole, 2003). Research shows that the plant roots naturally absorb pollutants, including such toxic chemicals as lead, mercury, and strontium 90% as well as some organic compounds (Never, 2015). It was observed that in aqueous solutions containing 5 mg/l of arsenic, chromium and mercury, the maximum uptake was 26 mg/kg, 108 mg/kg and 327 mg/kg of dry weight of water hyacinth, respectively (Ingole and Bhole, 2003). In addition to heavy metals, water hyacinth can remove toxic substances like cyanide, which is environmentally beneficial in areas with gold mining operations and is also used in Bangladesh to remove arsenic from contaminated drinking water (Misbahuddin and Fariduddin, 2002).

Because of its beautiful purple flower, water hyacinth can also be used as an ornamental plant. The flowers are pale purple, and one petal has a yellow spot on each flower. It is used as an ornament in outdoor pools and aquariums (Britannica, 2022). The value of additional aspects of floriculture-related products is even more difficult to determine (Bhattacharya and Kumar, 2010). Precise data on the various aspects of the water hyacinth as an ornamental plant is not available for different countries of the world, as a considerable amount of unrecorded economic activity takes place in local markets. (Anjanabha and Pawan, 2012).

Used as organic fertilizer, due to its high level of nutrient absorption capacity, the plant is rich in minerals. It can be used as an organic fertilizer, as a green manure or as compost (Ndimele, 2011; Never, 2015). As a green manure, it can be either ploughed into the ground or used as mulch. The research done by Gourba and Soumen (2008) recommends mixing chopped leaves and petiole of water hyacinth with cow dung in an 8:1 ratio (by volume), with a little bit of curd, may turn the water hyacinth into a good quality compost with good nutrient value in a short period of time. Research on turmeric crops done by Indulekha and Thomas (2018) suggested that mulching turmeric crops with plant materials including water hyacinth significantly improved their growth and yield attributes. When applied as mulch, water hyacinth contributed more available nitrogen and exchangeable potassium content to the soil compared to other mulch materials like jack and coconut leaves. In developing countries where mineral fertilizer is expensive, it is an elegant solution to the problem of water hyacinth proliferation and also poor soil quality (Never, 2015). However, its high alkalinity (pH $\geq$9) and potentially toxic heavy metals also contents restrict its use to flowering-plants, with no allowable application to horticulture for edible vegetables (Zhang, 2012; Never 2015).

Studies show that the nutrients in water hyacinth are good for the health of some animals like pigs, ducks, and pond fish by boiling chopped water hyacinth with rice bran, and vegetable waste (Jafari, 2010; Ndimele et al., 2011). The high water and mineral content mean that it is not suited to all animals, but it solves some of the nutritional problems that exist in developing countries.

Water hyacinth is used as an alternative fuel and energy source. It meets all of the criteria for bioenergy or ethanol production (Bote et al., 2020). It is perennial, abundantly available, non-crop plant, biodegradable, and has high cellulose (20%-40%) and lignin (10%) content (Cheng et al., 2015). Its strong disadvantage is that it has over 90% water content, which complicates harvesting and processing. Therefore, harvesting effort yields a low reward in terms of organic matter for conversion to biogas. The biomass can be subjected to biogas production to generate energy for household uses in rural areas (Bote et al., 2020). Mixing biomass of water hyacinth with pig manure leads to much higher biogas production than using pig manure alone (Lu and Wu, 2010).

Also, in small scale water hyacinth plants used in industrial and household articles, it has been used in several small cottage industries in the Philippines, Indonesia, and India. For paper, rope, baskets, mats, shoes, sandals, bags, wallets, and vases (Ndimele and Jimoh, 2011; Patel, 2012). Yet these are rarely successful at reducing infestations, and the market for these products is far too small to have any impact on water hyacinth populations. In addition, income generation may facilitate its spread to new, uninvited water bodies (Never, 2015).

2.6. Water Hyacinth's Natural Regrowth Potential

A water hyacinth is an aquatic free-floating perennial plant. Perennial plants have the ability to regrowth by vegetation, which means that even the smallest root or stem can reproduce an entire plant (Dhont et al., 2002; Vriet et al., 2014). The ability to regenerate after grazing or harvesting is an agronomic value trait in perennial plants. It is likely that the ability of plants to store and remobilize carbon and nitrogen reserves in persistent organs plays an important role in determining their perennial lifestyle and/or their capacity for vigorous regrowth following grazing, cutting back, or winter die-back (Vriet et al., 2014). Natural regeneration is a key component for securing the sustainability of perennial plants. It has the potential for the development of the next generation. The storage of carbon leads to the recovery of the ecosystem functions (Bullock et al., 2011).

For perennial herbaceous plants, Carbohydrate is the major storage compound and the carbohydrate reserve in root are believed to play an important role in resprouting plants (Bullock et al., 2011). New shoot in perennial plant has been directly associated with rapid depletion of carbohydrates in root. Starch is the most common storage form of carbohydrates in perennials plant (Zapata et al., 2004). In roots, the seasonal pattern of starch accumulation seems to differ both among species and among different site conditions (Von fircks and sennerby, 1998).

Water hyacinth roots have morphological plasticity, which is an important adaptive mechanism to acquire resources but the morphological plasticity of water hyacinth in roots is not well clarified (Xie and Yu, 2003). Water hyacinth only has first order lateral roots and forms a colossal root system with the root type of herring bone (one single main root with many laterals). The lateral roots have a root tip and may possess sufficient morphological plasticity in the root to exploit nutrients in low nutrient water bodies. (Xie and Yu, 2003). Root growth is important for uptake of storage of carbohydrates, and synthesis of growth regulators (Bullock et al., 2011). The root growth pattern of water hyacinth is genetically controlled but can be modified by the environment and the water nutrients (Xie and Yu, 2003).

The Perennial ties nature and the root character of water hyacinth make the plant more dangerous and uncontrollable (Gaikwad and Gavande, 2017). Knowing their nature is one of the first steps in controlling the growth and proliferation of the plant and disrupting the starch accumulation in the root by making the plant stressed and weak (Prased et al., 2008). It is one of the mechanisms to control the growth of water hyacinth and make the plant suitable for other control methods, like chemical, mechanical, and biological control methods**.**

2.7. Factors Affecting the Natural Regrowth Potential of Water Hyacinth

The regeneration potential of perennial plants is highly dependent on their carbohydrate accumulation and mobilization (Jing et al., 2020). Accumulation is influenced by internal and external factors. The major factors affecting the regrowth potential of plants after harvesting, wounding, grassing, or defoliation are seasons, climatic conditions, sunlight shading, nutrient concentration of the water, and water pH (Briske and Richards, 1995).

In wet seasons, the carbohydrate reserve is higher in summer (June, July and August) than in dry seasons because it is the time for maximum vegetation reproduction stage and flowering stages (Latt et al., 2001). But in this season, the biomass allocated to the root system decreased. The decrease in root system biomass allocation was perhaps because of the upward translocation of reserves to the new shoots or to support rapid growth and expansion (Ebrahem, 2018).

The nutrient concentration especially nitrogen affect the regrowth potential of water hyacinth after harvesting (Gaikwad and Gavande, 2017). Many river and lakes are rich in nitrates, phosphates and potassium because they receive polluted water from agriculture field, industrial zones and residential areas (Dersseh et al., 2019). For the optimum growth and regeneration the water field may contain the minimum nitrogen (20 mg L^{-1}), phosphate (3 mg L^{-1}) and potassium (53 mg L^{-1}) (De Groote et al., 2003). A favorable pH range for growth of water hyacinth is 6.5 to 8.5. It has been observed that regrowth of water hyacinth has decreased in both higher and lower values of the optimum range (Gaikwad and Gavande, 2017).

2.8. The Relationship between Nutrient Status and Water Hyacinth Growth Rate

Plants need nutrients to grow particular, nitrogen and phosphorus are important for the proliferation and production of water hyacinth (Mugidde and Wanda, 2002). Among other things, nitrogen is one of the major plant nutrients that can potentially limit the growth and nutrient uptake of water hyacinth (Reddy et al., 1989). Water hyacinth growth and multiplication rates were highly facilitated by the nutrient status of the water. Many studies have shown that the clonal growth of water hyacinth is correlated with the nutrient level of water bodies, especially with N and P (Mugidde and Wanda, 2002; Li x et al., 2015).

Although water hyacinth can grow well in clear lakes or with low nutrient availability, the proliferation and multiplication rates were slower than in nutrient rich lakes. It has also been observed to grow in extremely polluted waters containing rich organic matter, minerals, and heavy metals, as well as in waters with high acidity or alkalinity (Gopal, 1987). According to Malik (2007) the growth of water hyacinth plant in solution deficient of N, P and Ca were poor in fresh weight and in the number of normal leaves and also the number of new plants (daughter rament) was depressed and he conclude that water hyacinth is susceptible to N, P and Ca deficiency. Based on this, methods for controlling water hyacinth through minimizing water pollution by N and P have been suggested (Zulu et al., 2000).

Although a number of studies on water hyacinth control methods have been published in recent years, only a few of them have attempted to determine the optimal N concentration required to achieve maximum growth and multiplication. Minimizing pollution of water bodies from N and P, has not be successful alone in lake and river, because due to favorable nutrient environment. River flow and flush floods influence water hyacinth growth through continuous replenishment of nutrients (Mugidde and Wanda, 2002).

2.9. Nitrogen Fertilizer's Effect on Water Hyacinth Growth and Regrowth Potential

Water hyacinth's growth rate can be affected by water nutrient level, solar radiation, and season (Reddy and De Busk, 1984). Water hyacinth can take up large quantities of nitrogen, resulting in rapid growth and multiplication by developing strong cells for photosynthesis (Tham, 2012). Nutrient supply on growth and nutrient storage by water hyacinth plants has been widely reported. Increases in water nitrogen increase water hyacinth's growth rate (Reddy et al., 1989). Research indicates that nitrogen storage in the plant tissue increased with N supply rate, resulting in maximum N accumulation. Unlike the biomass yield, N storage in the plant tissue increased in proportion to N availability in the culture medium. The storage of N in the tissue is directly related to the biomass per unit area. (Reddy et al., 1989). Water hyacinth plants have the capacity to store nutrients in their tissue, but the stored nutrients in the tissue may not influence growth after a certain level of accumulation. (Reddy and DeBusk, 1987). The stored nutrients in the water hyacinth plant remain for a short time if the plant is not harvested. The dead tissue will decompose quickly and release nutrients into the water. Higher concentrations of nitrogen were accumulated in the shoots of Water hyacinth than in its roots.

Water hyacinth biomass, composition, and distribution can be used to explain seasonality in water hyacinth. In addition to the external nutrient inputs, internal nutrient loading due to the biological and physical re suspension promotes water hyacinth growth (Mugidde and wanda, 2002). In the shallow inshore regions of the lakes, wind re suspension of organic sediments returns nutrients to the water column. Organic sediments store and release nutrients to overlying waters more effectively than sandy sediments (Mugidde and Wanda, 2002).

Many papers show that the clonal growth of water hyacinth is highly related to the body's nutrient status, especially nitrogen and phosphorus (Bownes et al., 2013; Hill, 2014; Goa et al., 2016). For controlling water hyacinth, minimizing water pollination by N and P has been suggested (Zulu et al., 2000). Although water hyacinth grows well in clear lakes with low N and P availability, it has also been observed to grow in extremely polluted waters containing rich organic matter, minerals, and heavy metals, as well as in waters with high acidity or alkalinity (Gopal, 1987; Goa et al., 2016).

For control of water hyacinth, namely the reduction of nutrient inputs to the water is recommendable. Although strictly speaking, this is a preventative method, it can be argued that a reduction in nutrients in the water body will result in a reduction in the proliferation of water hyacinth. In recent decades, there has been a significant increase in the level of nutrients dumped into waterways from industrial and domestic sources as well as from land where fertilizers are used or where clearance has caused an increase in run-off (Singh and Gupta, 2016).

2.10. Effect of Harvesting Frequency on the Regrowth Potential of Water Hyacinth

Most researchers worked on how to control the water hyacinth's spread. But it is difficult to control aquatic free-floating invasive plants because flowing water can transport plants and their propagules (Tellez et al., 2008). There are a lot of control methods that are applied in the case of water hyacinth plants that are ineffective (Patel, 2012; Goa et al., 2016). Given current knowledge is very important to control of water hyacinth spread. To reduce its biomass and reproduction potential, to minimize the coverage area and to slow its rate of spread (Julien, 2008). To reduce or to control the invasion rate of water hyacinth in our eco systems, different types of control methods, including biological, chemical, and mechanical methods, have been released. However, until this day, those methods have had their negatives or have not been effective (Goa et al., 2016).

Water hyacinth's nutrient composition is generally related to nutrient availability in the habitat where the plants are growing. Water hyacinth grown in sewage has high protein and mineral content (Ho et al., 1994). According to Ansa and Garjila (2019), their research on elephant grass they says cutting frequency affects forage production, re-growth potential, and species survival among other factors like nutrient status of water; the plants get weak and thin out, probably as a result of a reduction in carbohydrate storage levels.

Water hyacinth photosynthesis rate highly affected by harvesting or by canopy removal. And this also altering resource allocation patterns and reducing relative growth rates (Trumble et al., 1993). Reduced root growth after harvesting is linked to the loss of photosynthetic tissue. Plant Carbon balance is modified in such a way that plant Carbon is preferentially allocated to shoots as a physiological adjustment in order to recover the leaf area lost (Genard et al., 2008).

Cutting height is an important consideration in the management of forage production systems, as it has crucial effects on regrowth rate, yield and quality, and persistence of forage plants (Shen et al. 2013). According to research, as harvest frequency increased, dry matter yield decreased. It has been well established that the productivity of forage plants is influenced by management factors, with cutting frequency being of major importance (Timpong et al., 2015).

Humphrey (2005) also indicated that lenient removal of grass shoots may still reduce net photosynthetic rate and cause unrecognized reductions in the movement of assimilate to the roots and thus reduce root growth. Additionally, Mealor (2010) reported that beyond 40% leaf volume removal, root growth by grass species begins to decline, and if the root system is disturbed, aboveground production also suffers. Cutting grasses between the third leaf and the reproductive stages can lead to beneficial responses, but cutting late in the seasonal development of the plant reduces the plant's ability to recover (Fulkerson and Donaghy, 2001).

2.11. The Effects of Nitrogen Fertilizer and Harvesting Frequency on Water Hyacinth Carbohydrate Reserves

Carbohydrates that are in the plant tissue can be divided into two main clusters: total structural carbohydrates (TSC) and total non-structural carbohydrates (TNC) (Eid, 2018). TSC includes permanent structural substances, such as cellulose, hemicelluloses, and other complex polymers. TSC provides the physical structure of the plant and usually remains where it is synthesized. In contrast, TNC can be converted to simple sugars and cycled in physiological processes. The TNC can be separated into two fractions: WSC (monosaccharide’s and disaccharides) and reserves (Eid, 2018).

Carbohydrate reserve theory states that the soluble carbohydrates stored in the roots and crowns of plants indicate plant health and the ability to regrow after harvesting or grazing (Madsen et al., 1993). Carbohydrate "reserves" are lower in the early vegetative stage of plant growth than in the late vegetative and early reproduction stages, so harvesting at the early vegetative stage is more effective but the plant cannot tolerate harvesting, whereas harvesting at the flowering or late vegetative stage is more effective and the plant can better tolerate harvesting (Chad, 2012).

Carbohydrate reserves are stored as starch. Carbohydrate resources accumulated in plant tissues (stems, roots, rhizomes, and stolons) that can be mobilized at a later date or after harvesting are used by the plant to provide energy and carbon for the survival and productivity of perennial plants (Loescher et al., 1990). They provide a source of carbohydrates for growth and metabolism during periods when the current photosynthetic supply of carbohydrates is not adequate to meet demands required to sustain normal organ growth and functioning (David et al., 2013).

The first thing for monitoring carbohydrate reserves was to show an index of potential regrowth based on the assumption that carbohydrates provided the major source for shoot growth (Briske and Richards, 1994). The depletion of carbohydrate reserves in response to harvesting, defoliation, environmental stresses, or as a result of normal phenological processes, was thought to be the major cause of growth reductions (Briske and Richards, 1994). In extreme cases, plant mortality could also result from a substantial reduction in carbohydrate reserves (Mitchell et al., 2013).

3. MATERIALS AND METHODS

3.1. Study Area Description

A pot experiment was conducted under shade house condition at College of Agriculture of Hawassa University during March – June, 2021. The area is located in Sidama region, southern Ethiopia, which is 273 km away from Addis Ababa, the capital city of Ethiopia. It is located at 7°3′N and 38°28′EC latitude and longitude, respectively with an elevation of 1,708 meters (5,604 feet) above sea level. The mean annual rainfall is about 971.9 mm, and the average temperature is 20.85 (National Meteorology Agency, 2020). The shade house used for the experiment was fenced with metal mesh wire while its roof was covered by transparent polycarbonate and

3.2. Materials for Experimentation

Water hyacinth plants used in the experiment were collected from the Koka Lake located in oromia region. Only newly produced ramets (stolon) of similar size (bearing 4–5 leaves) were chosen for the experiment. Each pot contained six healthy ramets.

3.3. Treatments and Experiment Design.

The treatment's constituted of different nitrogen concentration levels. The formulation of the growth solution consisted of a nitrogen fertilizer (urea) at different levels. Studies have shown that water hyacinth can tolerate high concentrations of nitrogen (Fox et al., 2008). It has also been shown that water hyacinth can absorb and store N and P in excess of what it requires for growth (hyper-accumulation or luxury uptake) (Alves et al., 2003). Based on water hyacinth's wide range of nutrient tolerance, we set up six levels of nitrogen fertilizer concentration (0 mg/l, 20 mg/l, 40 mg/l, 60 mg/l, 80 mg/l, 100 mg/l) and four harvesting frequency (un harvested, harvested once, harvested twice, and harvested thrice) were arranged in complete randomized design (CRD) in three replications. A total of seventy-two pots with six plants per pot were used, for a total of four hundred thirty-two plants. A uniform pot size of 40 cm in diameter and 18 cm in depth was used for each unit. During the experimental period, plants received tap water every 4 days along with the required cultural practice.

3.4. Experimental Procedures and Plant Management

Since the water hyacinth was primarily propagated by stolons, typically, the quality of stolons free from insects and diseases was selected visually directly from the field, from Koka lake Oromia region. Ten liter tap water was filled into the plastic pot, and different levels of nitrogen fertilizer were applied once on the planting date. Harvesting, which is removal of all the shoot parts above the root was started 15 days after planting. During the experimental period, the climate data (air temperature and relative humidity) were recorded. Since there was an occurrence of Fusarium wilt during the treatment periods, recommended rates of Power 76 WP chemical were applied. Harvesting is carried out in two intervals; once and twice harvesting treatments were done in the 15-day interval, and three times harvesting treatments were done in two interval the first two harvesting was done in 15 days interval and the third times harvesting was done at the 21-day after the second harvest. This time was selected to prevent the newborn leaves from photosynthesis activities. Thus, we ran the experiment for 4 months.

3.5 Analysis of Experimental Water Sampling

Experimental water samples prepared for laboratory analysis were taken from the tap water of College of Agriculture of Hawassa University. The samples were analyzed for their chemical properties (pH, electric conductivity, total dissolved soil, dissolved oxygen, macro and micro nutrients) using standard laboratory procedures at Hawassa University, Institute of Technology water supply and environmental engineering laboratory.

We measured the pH with a pH meter in the experiment pot. The electric conductivity (EC) of the experiment water was measured with a conductivity meter, and dissolved oxygen was measured with a dissolved oxygen meter. The method using membrane electrodes is a procedure based on the rate of diffusion of molecular oxygen across a membrane. Palintest Photometer, Round Test Tubes, 10 mL Glass (PT 515), with different Reagents used to measure chemical properties of experiment water following the manual. The Nitrite content of the experiment water was measured using Palindromic Nitricol Tablets. Palintest Nitratset Tube, 20 mL (PT 508), Palintest Photometer, Round Test Tubes, 10 mL (PT 515), watch with reagents: Palintest Nitricol Tablets are used to measure nitrate. Palintest photometer, round test tubes, 10 mL glass (PT 515), with reagents. Palintest Ammonia No. 1 Tablets and Palintest Ammonia No. 2 Tablets are used to measure ammonia, Reagents in round test tubes (10 mL glass) Palintest Alkaphot Tablets are used to measure the alkalinity of the water. 10 mL round test tubes (PT 515) are watched with the following reagents: Palintest Calcicol No. 1 and No. 2 Tablets are used to measure the calcium content of the water. Use 10 mL round test tubes (PT 515) and watch with reagents: Palintest Phosphate No. 1 & 2 LR Tablets are used for the measurement of phosphate levels, Palintest Photometer, Round test tube,10ml glass (PT 515), with reagent Palintest DPD NO1 and 3 tablets used to measure chlorine level (Fantahun, 2019)

Table 1. Physical and chemical properties of experimental water (tap water from Agriculture College)

Physical and chemical properties	Value	Rating	Reference
pH	8.17	good	Gaikwad and Gavande ,2017
E.C (µs)	1062	good	Casco et al., 2014
TDS(mg/l)	744	good	Casco et al., 2014
DO(mg/l)	7	good	Casco et al., 2014
Salt (ppm)	1062	good	Casco et al., 2014
Total nitrogen (mg/l)	1.07	Low	Gaikwad and Gavande ,2017
potassium (mg/l)	10.8	Low	Gaikwad and Gavande ,2017
phosphorous (mg/l)	0.51	Low	Gaikwad and Gavande ,2017
Sodium (mg/l)	84.1		
Calcium (mg/l)	80		
Iron (mg/l)	0		
F^- (mg/l)	1.49		
So_4^-(mg/l)	18		
Zn (mg/l)	0.0018		
Cl^- (Mg/l)	5.8		

3.6. Data Collection and Measurement

3.6.1. Meteorological Data

Temperature and Relative Humidity in the Air

On random selected days, data of air temperature and relative humidity were collected using Testo 174 mini data loggers (Testo 174, Version 5.0.2564.18771, Lenzkirch, Germany). The data logger was hanged closer to the plant canopy (30 cm above the ground) for each treatment and covered from the top with a flat carton to avoid direct sun and moisture. The pressure deficit of the shade house was calculated based on the temperature and relative humidity with VPD-Auto grow software (www.autogrow.com/wp content/uploads /2016/03/ VPD HDCALC.xls). The measurement was made every hour for 20 days.

3.6.2. Growth and Morphological Data

The data collection day was selected based on the water hyacinth growth rate, water hyacinth can bloom 3-4 weeks after planting in the hottest season (Aremu et al., 2012). This times was the maximum vegetative stage of water hyacinth, Due to this data collection was started at the 30 days after total harvesting done out.

Plant height (cm): the average plant height was measured from three randomly selected plants in each treatment, at 30 days after total harvesting done out.

Petiole diameter (cm): petiole diameter per plant was measured from three randomly selected plants in each treatment at 30 days after total harvesting done out, on maximum bulbous swelling area by using the Vernier Caliper instrument.

Branch number or leaf number (count): The branch number per plant was counted from three randomly selected plants in each treatment at 30 days after total harvesting done out.

New stolon or rament number (count): the number of new born stolon was counted from randomly selected three plants at the end of the treatment day.

Root length (cm): The length of the fibrous root per plant was measured for three randomly selected plants in each treatment at 30 days after total harvesting done out.

Root diameter (cm): At 30 days after total harvesting done out, root diameter was measured from three randomly selected plants in each treatment at a position where it was nearly cylindrical by meter.

Root number (count): The number of fibrous roots per plant was counted from three randomly selected plants in each treatment at 30 days after total harvesting done out

Leaf area per plant (cm^2): Leaf area was determined using a portable area meter model Ll-3000A belt-driven leaf area meter (LiCor Lincoln, Nebraska, USA) at 30 days after the start of the treatments, expressed in cm^2 $plant^{-1}$.

Leaf thickness (mm): leaf thickness was determined by using a vernier caliper instrument at 30 days after the start of the treatment, from randomly selected three plants in each treatment.

3.6.3 The Gas Exchange Parameters

Photosynthesis Efficiency (A), Transpiration Rate (E) and Stomatal Conductance (gs) Determination

The gas exchange parameters were measured from a young and fully expanded leaf at the 30- days after total harvesting done out. The data was taken from three randomly selected plants in each of the treatment using an open system LCA-4 (LCA-4 Software Version 1.04) ADC portable infrared gas analyzer (Analytical Development Company, Hoddeson, England). Were measured at 30 days after fully developed intact leaves. Measurement was done between 10:00 PM and 12:00 PM by maintaining the following specifications: the leaf surface area was 6.25 cm^2, the ambient carbon dioxide concentration was 386 µmol mol^{-1}, the leaf chamber mass flow rate was 251 µmol s^{-1}, and the atmospheric pressure was 840 bar and photosynthetic active radiation (PAR) were manually fixed to 600µmol m^{-2} s^{-1}.

Instantaneous Water Use Efficiency (A/E)

Instantaneous water use efficiency was determined as the ratio between net CO_2 assimilation rate (A) and transpiration rate (E) (Medrano et al., 2015). It refers to the balance between gains (kg of biomass produced or moles of CO_2 assimilated) and costs (m^3 of water used or moles of water transpired). It was calculated based on the data generated by CIRAS 3 portable photosystem (Version 2.01 110 Haverhill Road,Suite 301 Amesbury, MA 01913, U.S.A.) at 30 days after starting the treatment.

Relative Leaf Water Content (RLWC)

It was determined by the method stated by Chandrasekhar et al. (2000). At 30 days after total harvesting done out. That was, young fully expanded leaves were collected from each plot and sealed in the tubes. The tubes containing leaf samples were taken to the laboratory immediately after samples collected.

Leaf discs (9 mm in diameter) were cut out by using a cork disc cutter. Leaf discs that were cut from the leaves were directly weighed to determine their fresh weight (FW). There after the samples were immediately hydrated to full turgidity for 24 h by immersing in deionized water in a closed 15-ml tube under room temperature. The leaf samples were taken out of water and surface dried with tissue paper, and their turgid weights (TW) were recorded. The samples were packed in paper bag, and oven dried at 65 ^{o}C for 24 hours for dry weight (DW) determination. The leaf discs were weighed using an analytical balance with a precision of 0.00001 g. Then the calculation of leaf relative water content was computed as following the methodology of Turner (1981):

$$\text{LRWC (\%)} = \frac{FW - DW}{TW - DW}\, x100$$

Where FW denotes fresh weight, DW denotes dry weight, and TW denotes turgid weight.

3.6.4. Physiological Data

Determination of Chlorophyll Concentration

The contents of chlorophyll a (chl a), chlorophyll b (chl b), and total chlorophyll (TC) in the leaf tissues were assessed according to the method of Shabala et al. (1998). Leaf material was collected from the young and free from damaged leaves. For sampling of leaves, three leaves randomly selected from plants in each treatment were used, covered with aluminum foil and transported to the laboratory of crop physiology. For the extraction, about 0.2g of fresh leaf samples was placed in a 15-ml glass vial with 10 ml of 95% alcohol. The glass vials were sealed with Para film to prevent evaporation and chlorophyll degradation by light. The homogenized sample mixture was centrifuged for 15 minutes at 40°C at 10,000 rpm. The supernatant was separated and 0.5 mL of each concentration level was analyzed in a laboratory dark room for Chlorophyll a and Chlorophyll b at an absorbance of 649 nm and 664 nm wavelength region using a UV-2450 spectrophotometer (Hitachi, Tokyo, Japan). The following equations were used for the quantification of Chlorophyll a, Chlorophyll b, and total chlorophyll (Lichtenthaler and Buschman, 2001).

Chl a (μg/ml) =13.36 (A664)–5.19 (A649)

Chl b (μg/ml) = 27.43 (A649) - 8.12 (A664)

TC (μg/ml) = chl a + chl b

Where; A = Absorbance, Chl a = Chlorophyll a, Chl b = Chlorophyll b, TC = Total Chlorophyll

3.6.5 Parameter for the Dry Matter Component

Shoot Fresh Weight (gm.):- It was measured using sensitive balance at all the harvesting times and at the end of the treatment from all shoot of three selected plants per experimental unit.

Root Fresh Weight (gm.):- It was measured using an electro sensitive balance at the end of harvesting and at the end of the treatment from three selected plants per experimental unit.

Total biomass (gm.): it is a sum of the fresh weight of shoot and the fresh weight of root per plant.

Shoot Dry weight (gm.):- it was measured by an electronic sensitive balance after the samples had been oven-dried (48 hrs. at 75°C).

Root Dry Weight (gm.):- it was measured by electronic sensitive balance after the samples had been oven-dried (48 hrs. at 75°C).

Total Dry Matter: It is the sum of shoot dry mass and root dry mass per plant.

3.7. Data Analysis

The effects of nitrogen fertilizer and harvesting frequency on the growth, physiological responses, leaf gas exchange, and dry mater component of water hyacinths were analyzed using a repeated measure ANOVA with a post hock Tukey test by SAS version 9.3 (SAS Institute, 2018). The Tukey's HSD test was used to separate means at the 5% level of significance.

4. Results and Discussion

4.1. Meteorological Data

4.1.1 Deficit in Air Temperature, Relative Humidity, and Vapour Pressure

The maximum and minimum air temperature of the shade house was recorded at 11:18:00 am and at 5: 18 am was 32.94 ℃ and 13.20℃, respectively, (Table 2). The shade house temperature condition was in the range of optimum temperature (22.08 ^{o}C), which is good for the growth and multiplication of water hyacinth. Research shows that the optimum temperature for water hyacinth growth was range from 25-30 ^{o}c. but it also have high air temperature tolerance capacity minimum up to 12^{o}c and the maximums goes to 35 ^{o}c (Minychl and Dessalegn, 2019).

Regarding relative humidity, the maximum daily relative humidity 93.33% was recorded at 5:18:00 am and the minimum 34.23% was recorded at 11:18:00 am the mean relative humidity was 62.26%. The recommended shade house relative humidity is between 40%- 70% (Shamshiri et al., 2018). Our shade house environment related to relative humidity is good for the growth of water hyacinth (Table 2).

Regarding vapor pressure, the maximum vapor pressure difference was 2.90 KPa recorded at 12:18 pm and the minimum was 0.10 KPa recorded at 5:18 am. The mean vapour pressure of the shade house was 0.19 KPa (Table 2), the recommended vapour pressure for plant was between 0.5- 1.2 KPa (Shamshiri et al., 2018).

In general, our experimental shade house environment are suitable for the growth and proliferations of water hyacinth.

Table 2. Shade house climatic data during the experimental period (average of 20 days)

Hours	Temperature ^{o}C	RH	VPD (kPa)
12:18:00 PM	32.95	42.18	2.90
1:18:00 PM	31.27	46.15	2.46
2:18:00 PM	29.10	40.8	2.39
3:18:00 PM	28.64	45.86	2.12
4:18:00 PM	26.4	45.93	1.86
5:18:00 PM	22.03	60.7	1.04
6:18:00 PM	20.7	66.36	0.82
7:18:00 PM	19.67	69.12	0.7
8:18:00 PM	18.25	75.16	0.52
9:18:00 PM	18.05	74.28	0.53
10:18:00 PM	17.17	78.12	0.42
11:18:00 PM	16.95	79.96	0.39
12:18:00 AM	16.28	82.15	0.33
1:18:00 AM	15.87	84.13	0.28
2:18:00 AM	15.05	83.53	0.29
3:18:00 AM	14.92	87.87	0.2
4:18:00 AM	13.50	91.86	0.13
5:18:00 AM	13.20	93.33	0.10
6:18:00 AM	17	81.67	0.33
7:18:00 AM	20.5	69.95	0.72
8:18:00 AM	26.6	54.83	1.57
9:18:00 AM	29.7	46.65	2.22
10:18:00 AM	31.80	39.50	2.84
11:18:00 AM	34.35	34.23	3.57

4.2. Morphological and Growth Parameters Response for Nitrogen Fertilizer and Harvesting Frequency

In this study, the morphological responses of water hyacinth to nitrogen fertilizer and harvesting frequency were determined based on the plant height, petiole diameter, leaf number, leaf thickness, leaf area, stolon number, root length, root number, and root diameter. The analysis of variance revealed that the interaction effect of nitrogen fertilizer and harvesting frequency had a significant influence on morphological parameters. These results indicated that nitrogen fertilizer has an effect on the growth, development and regrowth potential of water hyacinth, and also harvesting frequency affects the growth and the regrowth potential of water hyacinth. Detail descriptions are present as follows:

4.2.1 Plant Height, Petiole Diameter, and Stolon Number

Analysis of variance revealed that significant influences on plant height, petiole diameters, and new born stolons were observed due to the interaction effect of nitrogen fertilizer and harvesting frequency (Appendix Table I). Plant height of water hyacinth was significantly ($p \leq 0.01$) influenced by the main effect of nitrogen fertilizer and harvesting frequency, and by the interaction effect of nitrogen fertilizer and harvesting frequency (Table 3).

According to the results, the tallest plant was observed from 60 mg L^{-1} nitrogen treated with un harvest, and the shortest plant was observed at 0 mg L^{-1} and 100 mg L^{-1} nitrogen treated with harvested thrice (Table 3). This research revealed that plant height were highly affected by the nitrogen fertilizer up to 60mg L^{-1} the water hyacinth have good respond for nitrogen fertilizer in its growth but the concentration of nitrogen increase it doesn’t influence the growth of water hyacinth. This was related to Reddy and Debusk (1987) who were revealed that water hyacinth plants have the capacity to tolerate excess nutrients and store nutrients in their tissue, but the stored nutrients in the tissue may not influence growth after a certain level of accumulation. The harvesting frequency and their interaction effect were also revealed. Unharvest treatment has the maximum height growth. Increasing harvesting frequency may affect the shoot growth of water hyacinth. In general, when harvesting increased, the plant became more stressed, and stress due to harvesting induced habitual growth of water hyacinth. This effect was observed in the growth of water hyacinth, especially in plant height.

Petiole diameter was also significantly ($p \leq 0.01$) affected by nitrogen fertilizer, harvesting frequency, and their interactions. The swollen petiole was observed from 100 mg L^{-1} nitrogen treatment and followed by 0 mg L^{-1} nitrogen treatment with un harvest , and the thin petiole was observed from the 60 and 40 mg L^{-1} nitrogen treated with harvested thrice (Table 3).

Mostly water hyacinth petiole became swallowed in low- nutrient and excess- nutrient area and it was a morphological adaptation character of water hyacinth response for unsuitable environment and the swallow's petiole contains air; that air helps the plant float in water (Julien et al., 2001). And also as harvesting frequency increase their was high decrease of petiole diameter observed in all nitrogen treatment. The tiny or slender petiole is may be character of suitable environment and the plant need to stay in that environment. The morphology of their petioles is one of the best mechanisms for the water hyacinth to adapt to environmental change. (Julien et al., 2001)

Table 3. The interaction effect of nitrogen fertilizer and harvesting frequency on plant height and petiole diameter of water hyacinth.

	Nitrogen Fertilizer level (mg L^{-1})					
Harvesting	0	20	40	60	80	100
Plant height						
Un harvested	18.64^{fgh}	32.2^{bc}	33.67^{ab}	36.57^{a}	26.64^{d}	16.5^{hi}
Harvested once	15.23^{hijk}	21.65^{efg}	29.64^{cd}	29.21^{cd}	21.23^{efg}	14.96^{hijkl}
Harvested twice	15.0hi	18.16^{fgh}	22.67^{e}	21.88^{ef}	15.41^{hij}	13.46^{ijkl}
Harvested thrice	11.50^{l}	11.70^{jkl}	16.68^{hi}	18.05^{gh}	13.20^{jkl}	11.36^{l}
Mean		20.24				
LSD (0.05)		3.74				
CV %		11.26				
Petiole diameters						
Un harvested	13.43^{b}	12.54^{cd}	9.76^{gh}	8.54^{i}	12.56^{cd}	14.63^{a}
Harvested once	12.20^{df}	10.60^{f}	7.46^{j}	8.81^{i}	11.70^{e}	13.12^{bc}
Harvested twice	9.22^{hi}	7.41^{j}	4.85^{l}	4.57^{lm}	4.63^{l}	10.48^{fg}
Harvested thrice	6.08^{k}	4.98^{lm}	3.81^{mn}	3.25^{n}	3.36^{n}	6.13^{k}
Mean		8.5				
LSD (0.05)		0.81				
CV %		5.83				

Means sharing the same letter in a column of treatment are not statistically significant at the 95% confidence level based on the LSD (Least significance difference) comparison method.CV=coefficient of variance

The stolon production also significantly ($p \leq 0.01$) influenced by nitrogen fertilizer, harvesting frequency as well as their interaction effect of nitrogen fertilizer and harvesting frequency.

From our result 60 mg L^{-1} nitrogen fertilizer treated with harvested once produced the more new born stolon, while the 0 mg L^{-1} and 100 mg L^{-1} nitrogen fertilizers treated with harvested thrice treatment had fewest stolons (Table 4).

In the stolon production, a higher value was observed from the harvested once treatment with 60 mg L^{-1} N. and also at harvested once, the highest stolon production was observed in all nitrogen treatment. This result indicates that at the first harvest or shoot removal, the water hyacinth used its energy (the stored carbohydrates in its root) with the help of nitrogen fertilizer to face the stress by producing new stolons quickly and abundantly (Julien et al., 2001). But as the harvest level increased, the number of newborn stolons sharply decreased at all nitrogen levels (Table 4). Maybe this declaration is related to the continued stress that came from harvesting, which affects the stored carbohydrates and the capacity of the plant to regenerate by producing stolons. By nature, water hyacinths have the capacity to replace themselves quickly and produce more stolons. Stolon production was related to suitable environmental conditions, especially with nitrogen (Zhang and Guo, 2017). Knowing their nature is one of the first step in controlling the growth and performance of water hyacinth. From our experiment results, the integration of reducing the water nutrients and frequent harvesting is more effective in the control of water hyacinth spread.

Table 4. Number of new stolon growth of water hyacinth influenced by the interaction effect of nitrogen fertilizer and harvesting frequency

	Nitrogen fertilizer levels (mg L^{-1})					
Harvesting	0	20	40	60	80	100
Un harvested	1.78^{ef}	1.99^{de}	2.88^{b}	3.10^{ab}	2.20^{cde}	1.42^{fgh}
Harvested once	2.21^{cd}	2.42^{c}	3.20^{ab}	3.45^{a}	2.20^{cde}	1.99^{de}
Harvested twice	1.55^{fg}	1.78^{ef}	2.20^{cde}	2.10^{cde}	1.20^{ghi}	1.20^{ghi}
Harvested thrice	0.55^{j}	1.22^{gh}	1.00^{hi}	1.20^{ghi}	0.78^{ij}	0.54^{j}
Mean		1.84				
LSD(0.05)		0.43				
CV%		14.18				

Means sharing the same letter in a column of treatment are not statistically significant at the 95% confidence level based on the LSD (Least significance difference) comparison method. CV=coefficient of variance

4.2.2 Leaf Number, Leaf Thickness and Leaf Area

The main effects of nitrogen fertilizer and harvesting frequency, as well as the interaction effect, influenced the leaf number of water hyacinth significantly (P< 0.001) (Appendix Table I).

The maximum leaf number was recorded from treatment 60 mg L^{-1} nitrogen treated with unharvest, and the minimum leaf number was observed from 0, 80, 100 mg L^{-1} nitrogen treated with harvested thrice. The leaf number of water hyacinth sharply dropped under harvesting frequency by 69%, 73.4%, 72.1%, 67.2%, 76.1%, and 70.5% when nitrogen fertilizer at 0, 20, 40, 60, 80 and 100 mg L^{-1}, respectively (Table 5).

Leaf number is highly related to nitrogen fertilizer. Increasing the concentration of nitrogen up to 60 mg L^{-1} increases the plant leaf count but after 60 mg L^{-1} the nitrogen level doesn't increase the leaf number. Sufficient nitrogen level encourages vegetative growth, especially leaf number. This is what we see in our experiment: water hyacinth grow at low nutrient levels, but for rapid growth and multiplication, and developing strong cells for photosynthesis, it needs sufficient nitrogen (Tham, 2012).

And also harvesting frequency had direct effect on the leaf number of water hyacinth, as we see from the result harvesting increase leaf number was sharply decrease in all nitrogen levels (Table 5). This result was related to another studies on Amaranth plant, it was indicated that frequent harvesting reduced the number of leaves per plant in Leaf Amaranth (Dinssa et al., 2018).

Leaf thickness was significantly ($P \leq 0.001$) affected by the main effects of nitrogen fertilizer and harvesting frequency, as well as the interaction effect (Appendix Table I).

From our experiment, 20 mg L^{-1} treated with un harvest had the thicker leaves. While 100 mg L^{-1} treated with harvested three times had the thinner leaves (Table 5). From this finding, excess nitrogen fertilizer level in the water has an effect on the leaf thickness of water hyacinth. Nitrogen fertilizer level increase resulted in reduction of leaf thickness. Leaf thickness was reduced under harvesting frequency by 40.5%, 35.4%, 32.3%, 33.8%, 34.5% and 31.25% on the nitrogen fertilizer levels 0, 20, 40, 60, 80 and 100 mg L^{-1} when, respectively (Table 5). As harvesting frequency increases from unharvest to harvest three times the leaf thickness was highly reduced in all treatments. Water hyacinth leaves contain air filled parenchyma tissue which gives the plant its considerable buoyancy and help it float in the water (Julien et al., 2001). As harvesting increase the water hyacinth leaf loss their buoyancy capacity and the morphological adaptation for the survival on water bodies. This is due to harvesting stress and it makes the plant week.

Table 5. Interaction effect of nitrogen fertilizer and harvesting frequency on leaf number and leaf thickness (mm) of water hyacinth plant

	Nitrogen fertilizer levels (mg L^{-1})					
Harvesting	0	20	40	60	80	100
Leaf number						
unharvested	7.53^{fg}	10.66^{d}	11.33^{cd}	13.00^{a}	9.77^{e}	7.89^{f}
Harvested once	7.63^{fg}	10.88^{cd}	12.22^{b}	11.40^{c}	9.55^{e}	7.11^{g}
Harvested twice	4.21^{j}	5.63^{hi}	5.73^{h}	7.86^{f}	5.00^{i}	4.00^{j}
Harvested thrice	2.33^{l}	2.83^{kl}	3.16^{k}	4.26^{j}	2.33^{l}	2.33^{l}
Mean		7.02				
LSD (0.05)		0.72				
CV %		6.29				
Leaf thickness (mm)						
unharvested	0.74^{b}	0.79^{a}	0.68^{cd}	0.65^{de}	0.58^{fg}	0.48^{ijk}
Harvested once	0.70^{bc}	0.70^{bc}	0.63^{ef}	0.52^{hi}	0.40^{mno}	0.38^{nop}
Harvested twice	0.53^{h}	0.62^{ef}	0.54^{gh}	0.46^{ikl}	0.41^{mon}	0.35^{pq}
Harvested thrice	0.44^{klm}	0.51^{hij}	0.46^{kj}	0.43^{lmn}	0.38^{op}	0.33^{q}
Mean		0.53				
LSD (0.05)		0.04				
CV%		5.07				

Means sharing the same letter in a column of treatment are not statistically significant at the 95% confidence level based on the LSD (Least significance difference) comparison method. CV=coefficient of variance

Total leaf area per plant was significantly ($P \leq 0.01$) affected by the main effects of harvesting frequency and nitrogen fertilizer level as well as the interaction effect (Appendix table II). The maximum leaf area was observed from un harvest treatment treated with 40 and 60 mg L^{-1} N, whereas the minimum leaf area was observed from harvested thrice treated with 0 mg L^{-1} N treatment (Table 6). Leaf area reduced sharply as harvesting increased from unharvested to harvested three times by 79.7%, 73.8%, 75.3%, 67.16%, 74.7%, and 70.7% when 0, 20, 40, 60, 80 and 100 mg L-1 were applied respectively (Table 6).

According to our findings, 40 and 60 mg L^{-1} N are the best for water hyacinth leaf growth. Up to 60 mg/L^{-1}, water hyacinth leaf growth responded positively to N fertilizer, but further increases in N supply rate did not increase water hyacinth leaf growth, whereas harvesting increased leaf growth of water hyacinth decrease. This may be due to the stress that came from harvesting inhabits leaf expansion, cell enlargement, or cell division or it may be the mechanism to adopted the stress by reducing leaf area, that help in redaction of transpiration and photosynthesis. Plant growth, particularly leaf area, is highly related to nitrogen fertilizer; nitrogen promotes healthy leaf growth by stimulating chlorophyll production. Reduction of leaf area is positively related to stomatal and chlorophyll reduction, those redaction have direct effect on carbohydrates production (starch) that help the water hyacinth regenerate after harvesting. Leaf area redaction have indirect effect on regrowth potential of water hyacinth. (Bhagsari and Brown, 1986).

Table 6. Leaf area (cm^2) of water hyacinth plant influenced by the interaction effect of nitrogen fertilizer and harvesting frequency

Harvesting	**Nitrogen fertilizer levels(mg L^{-1})**					
	0	20	40	60	80	100
Unharvested	326.40^{fgh}	470.24^{bc}	580.52^{a}	609.71^{a}	429.73^{cd}	332.81^{fg}
Harvested once	281.61^{hi}	402.66^{de}	480.19^{b}	460.71^{bc}	357.16^{ef}	289.10^{ghi}
Harvested twice	183.70^{kl}	315.53^{fghi}	277.67^{ij}	359.63^{ef}	232.24^{jk}	143.31^{lm}
Harvested thrice	66.27^{mn}	123.20^{m}	143.31^{lm}	200.17^{k}	108.77^{mn}	97.33^{mn}
Mean		303.02				
LSD (0.05)		48.56				
CV %		9.74				

Means sharing the same letter in a column of treatment are not statistically significant at the 95% confidence level based on the LSD (Least significance difference) comparison method.CV=coefficient of variance

4.2.3 Root Length, Root Number, and Root Diameters

The main effects of nitrogen fertilizer and harvesting frequency, as well as the interaction effect had influenced the root length of water hyacinth significantly ($P< 0.001$) (Appendix Table II).

The tallest root was recorded (36.85cm) from 0 mgL^{-1} N treated with un harvest ; whereas the shortest roots were recorded (16.73) from 100 mg L^{-1} N treated with harvested thrice (Table 7). Root length was significantly reduced under harvesting and under higher N solution treatment. Furthermore, there was no significant difference between the 0 mg L^{-1} and 20 mg L^{-1} N treatment with harvesting frequencies of one, two, and three times on root length. From our findings, treatments of 0 mg L^{-1} and 20 mg L^{-1} had higher harvesting tolerance than the other four treatments on root length, and the result also tells us that root growth had a reverse relation to nitrogen fertilizer levels; a low nitrogen level encourages root elongation and root growth. By nature, water hyacinths have long roots, but with low nutrients, the growth of the root increases to absorb nutrients, short root is the character of sufficient nutrients in the environment (Xie and Yu, 2003). Our results show that harvesting frequency had no effect on root length of water hyacinth at low nitrogen concentration levels (in 0 and 20 mg L^{-1}).

Table 7. Root length (cm) of water hyacinth plant influenced by the interaction effect of nitrogen fertilizer and harvesting frequency

	Nitrogen fertilizer levels (mg L^{-1})					
Harvesting	0	20	40	60	80	100
unharvested	36.85[a]	30.47[b]	24.19[c]	21.56[d]	21.21[de]	26.22[c]
Harvested once	30.06[b]	30.96[b]	24.55[c]	20.20[def]	30.55[b]	21.61[d]
Harvested twice	30.71[b]	30.57[b]	21.73[d]	20.63[de]	19.30[def]	19.74[def]
Harvested thrice	30.02[b]	29.46[b]	20.05[def]	19.73[def]	18.13[ef]	16.73[g]
Mean		24.54				
LSD (0.05)		2.09				
CV %		5.11				

Means sharing the same letter in a column of treatment are not statistically significant at the 95% confidence level based on the LSD (Least significance difference) comparison method.CV=coefficient of variance

Root diameter was significantly ($p<0.001$) affected by the main effects of nitrogen fertilizer and harvesting frequency as well as the interaction effect (Appendix II).The largest root diameter was observed at 0 mg L^{-1} N treated with unharvested, and the smallest root diameter was observed at 100 mg L^{-1} N treated with harvested thrice (Table 8). This result indicated that root diameter was affected by nitrogen fertilizer but was highly affected by harvesting. When harvesting increases, the diameter of the root greatly decreases. When a water hyacinth shoot is harvested, the plant becomes stressed, and the root size decreases. The carbohydrate reserve in the root was believed to play an important role in reporting plants (Loescher *et al., 1990*). As harvesting increases, more dry matter may be depleted from the root part to regenerate new shoots, resulting in a significant reduction in root diameter, which also implies a reduction in root regeneration potential. Lateral roots of water hyacinth were longer and denser at low-N fertilizer treatments than at high-N fertilizer treatments. (Reddy and Sutton, 1984) The diameter also increased when grown in conditions with low-N fertilizer availability.

Root number was significantly ($p<0.001$) affected by the main effect as well as the interaction effect of nitrogen fertilizer and harvesting frequency (Appendix Table II). The highest root number was (68.66) recorded at 0 mg L^{-1} with un harvested treatment and the smallest root number (24.76) was observed at 0 mg L^{-1} nitrogen treated with three times harvest. The result showed that harvesting has a high effect on root number. When harvesting increases there is a significant reduction on the root number was observed by 63.9%, 55.5%, 56%, 34.5%, 59%, 57.9% in treatment '0mg/l', '20mg/l', '40mg/l', '60mg/l', '80mg/l', and ‘100mg/l’ N fertilizer respectively (Table 8). As discussed above, nitrogen concentration level have reverse effect root growth but harvesting frequency had a direct effect on root number of water hyacinth. The results showed that frequent harvesting stresses the water hyacinth, and as stress increases, the water hyacinth decrease in root number, which is one of the stress-response mechanisms in aquatic plants (Doyle, 2001). The highest and the lowest root number was observed from 0 mg L^{-1} N treatment, this means high reduction was on 0 mg L^{-1} treatment. And harvesting tolerance regarding root number was observed in the 60 mg L^{-1} N treatments. Root growth was high at low nitrogen levels (0 mg L^{-1}), but the root at low nitrogen levels cannot tolerate harvesting frequency in the root number, as compared to 20, 40, 60, 80, and 100 mg L^{-1}. For regrowth after harvest, the stored carbohydrate in the root is essential, when the root drops, it means it loses the reserve carbohydrate and the capacity to regrowth after harvest (Latt et al., 2000). In high nitrogen concentrations, especially at 60mg/l the drop of the root is low and has the capacity of harvesting tolerance in root number, which means it has good regeneration capacity as compared to the other levels.

The results indicated that the variation in root of water hyacinth can be considerable and the changes in root morphology depend on the amount of available N fertilizer and harvesting frequency. In a high nutrient environment, maybe lateral root growth was inhibited by the high N concentration in the plant (Xie and Yu, 2003). Robinson and Van (1998) found that fast-growing species exhibit, on average, a higher level of root morphological plasticity than slow-growing species in their paper. These variations could be the result of various growth rates (Fransen et al., 1999). The water hyacinth has one of the highest growth rates of any plant that is currently known, therefore a high productivity rate may be the cause of the lateral roots' extraordinary morphological plasticity.

Our study's findings demonstrated that morphological changes account for the diversity in roots' length, number, and density. It is advantageous for water hyacinths to adapt to a low N fertilizer environment by growing long, thin roots (Xie and Yu, 2003). However, harvesting can have an impact on morphological adaptation since it drastically reduces the number and density of water hyacinth roots. This decline also denotes a decrease in the amount of stored carbohydrates and a reduction in the water hyacinth's ability to regenerate following harvest.

Table 8. Root diameter (cm) and root number of water hyacinth plant influenced by the interaction effect of nitrogen fertilizer and harvesting frequency

	Nitrogen fertilizer levels (mg L^{-1})					
Harvesting	0	20	40	60	80	100
Root diameter (cm)						
Unharvested	31.26^{a}	26.99^{c}	22.28^{fg}	25.53^{cd}	28.87^{b}	30.41^{ab}
Harvested once	29.05^{b}	24.96^{de}	21.35^{gh}	21.94^{fgh}	23.16^{f}	25.46^{cd}
Harvested twice	23.40^{ef}	20.52^{hi}	19.44^{i}	21.19^{gh}	15.15^{j}	19.04^{i}
Harvested thrice	12.67^{k}	15.39^{j}	15.66^{j}	18.86^{i}	14.70^{j}	10.72^{l}
Mean		21.58				
LSD (0.05)		1.66				
CV %		4.69				
Root number						
Unharvested	68.66^{a}	63.40^{b}	59.41^{c}	57.06^{cd}	65.30^{b}	64.43^{b}
Harvested once	56.20^{cd}	54.30^{de}	55.55^{de}	45.06^{e}	52.75^{e}	55.44^{de}
Harvested twice	42.63^{g}	45.06^{fg}	46.55^{f}	41.97^{g}	36.08^{h}	34.55^{h}
Harvested thrice	24.76^{j}	28.20^{i}	26.11^{ij}	37.40^{h}	26.77^{ij}	27.10^{ij}
Mean		46.45				
LSD (0.05)		3.28				
CV %		4.30				

Means sharing the same letter in a column of treatment are not statistically significant at the 95% confidence level based on the LSD (Least significance difference) comparison method.CV=coefficient of variance

4.3. Physiological parameter response to nitrogen fertilizer and harvesting frequency

Analysis of variance revealed that the gas exchange parameters [photosynthetic rate (μmol m^{-2} s^{-1}), transpiration rate (mmol m^{-2} s^{-1}) and stomatal conductance (molm^{-2} s^{-1})] were significantly affected by the main effects as well as the interaction effects of nitrogen fertilizer and harvesting frequency (Appendix V).

4.3.1. Photosynthetic (A) μmol m-2s-1

Analysis of variance revealed that there was a significant difference in the photosynthetic rate ($p<0.05$) affected by the main effect and the interaction of nitrogen fertilizer and harvesting frequency. The maximum photosynthesis was observed at 60 mg L^{-1} N, followed by 40 and 20 mg L^{-1} treated with unharvest. However, photosynthesis efficiency significantly decreases with increasing harvesting frequency at all nitrogen fertilizer levels. The minimum photosynthesis was observed at 0 and 100 mg L^{-1} N with harvested thrice (Figure 1). This indicates that nitrogen fertilizer has a direct effect on photosynthesis rate above 60 mg L^{-1} N and below 60 mg L^{-1} N, the rate of photosynthesis significantly decreases. The study also showed that when harvesting frequency increased, the photosynthesis efficiency of water hyacinth significantly decreased. This reduction may be due to stomata closure and may be due to higher temperature and higher VPD during the growth period (Table 2) (Urban et al., 2017).

This reduction in photosynthesis rate might be used as a strategy to coping from the stress that came from shoot harvesting frequency. In photosynthetic reactions, molecular oxygen is involved as a catalyst to facilitate the assembly of energy compounds, leading to the production of glucose. The production of glucose stored in the root can help the plant to regenerate after harvesting. However, under a variety of stress conditions, plants reduce photosynthesis efficiency, growth and development as an avoidance strategy. Under stress conditions, leaf area, leaf thickness and chlorophyll content reduced and all this factors are contribute to a decrease in photosynthetic rate (Wolfe et al., 1988). The capacity of nitrogen in shoot growth, namely leaf number and leaf area, is similarly impacted by harvesting frequency. A significant drop of photosynthesis was see as the sufficient amount of nitrogen level as harvesting increased from unharvest to thrice harvest.

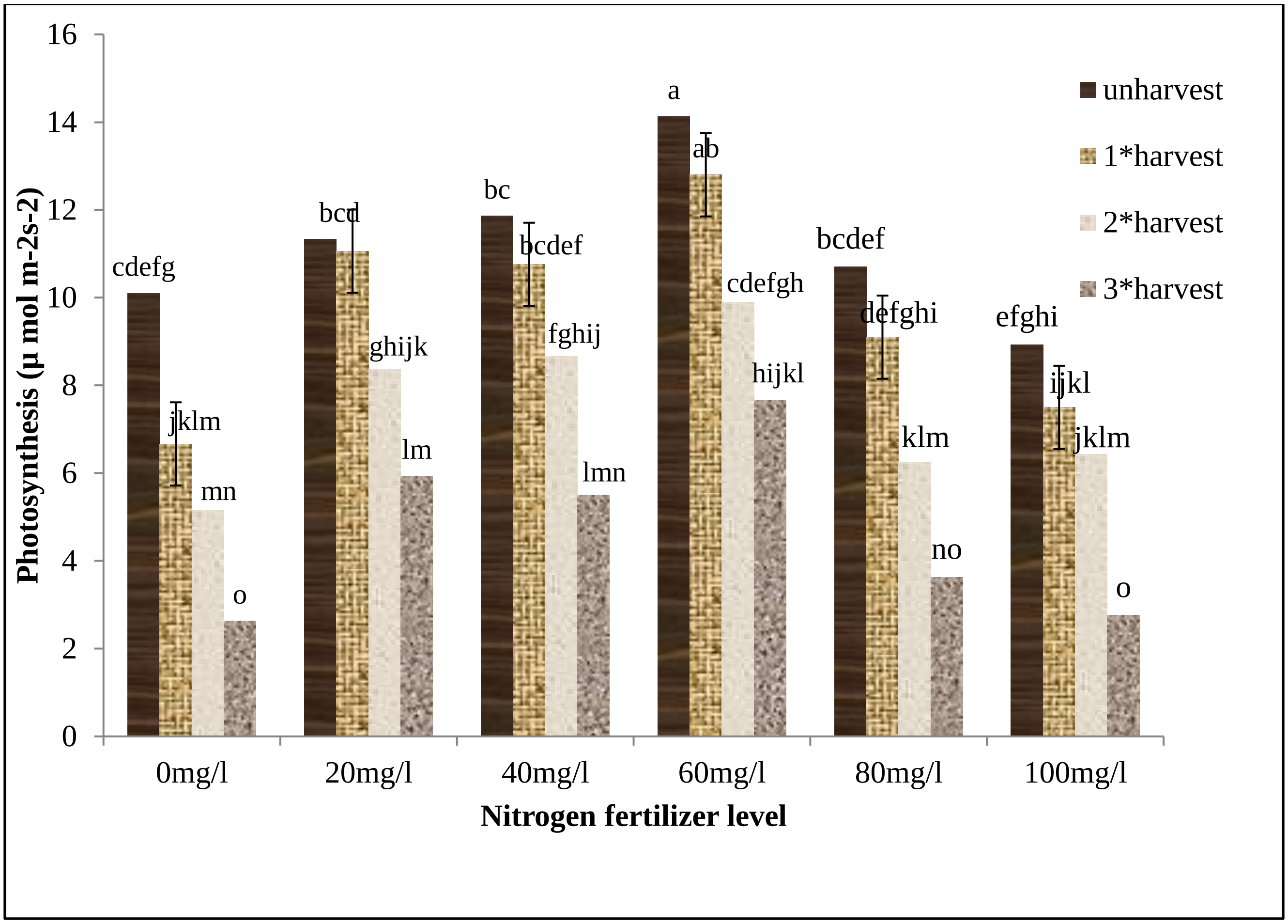

Figure 1. Interaction effect of nitrogen fertilizer and harvesting frequency on photosynthesis rate. While, means with different letter on the figure are statistically significant at P-values values < 0.05, LSD (0.05), = 2.24 and CV (%) = 16.59.

4.3.2. Transpiration (E) mmol m-2s-1

The study revealed that transpiration was significantly ($P < 0.01$) affected by the main effects of nitrogen fertilizer and the interaction of nitrogen fertilizer and harvesting frequency, but harvesting frequency had no significant ($P > 0.05$) effect on the transpiration rate of water hyacinth. The maximum transpiration rate was observed from 60mg/l N treated with harvested once (12.53) and the minimum transpiration rate was observed from the treatment of 100mg/l N fertilizer treated with harvested thrice (3.19) (Figure 2). The result indicates that the transpiration rate of water hyacinth leaves depends on nitrogen fertilizer. The concentration of nitrogen in the waters increase leaf number and leaf area also increased. Transpiration rate have correlated with leaf number and leaf area. The leaf area and leaf number influences the rate of transpiration and stomata number. The wider the leaf area, the greater the rate of transpiration because wide leaves tend to have more stomata (Maylani et al., 2020). Nitrogen deficiency increases stomatal resistance and reduces transpiration (Da Silva et al., 2011).

Perhaps, water loss from plant shoot increases the vapor pressure gradient between the ambient air and leaf and consequently increased the transpiration rate. However, a significant decline in the transpiration rate was observed from frequent harvested treatment this is directly related to redaction of leaf growth Water loss due to water hyacinth can reach three times greater than the natural evaporation rate of a water surface that does not have water hyacinth (Maylani et al., 2020).The reduction in transpiration might be related to a reduction in stomata conductance that causes a reduction in gas exchange due to stomatal movement (Pirasteh et al., 2016). Stomata are the main channels for leaves to exchange water and gas with the environment. In the main photosynthetic organs, they are closely related to plant physiological activities such as photosynthesis, transpiration, and respiration.

The process of transpiration is also influenced by internal factors like leaf size, leaf thickness, stomata number, and location of stomata. From our research investigation, the higher leaf area was observed from the 60mg/l N treatment and also the maximum transpiration was observed on that treatment (Figure 2). A high rate of transpiration would be conducted to increase growth.

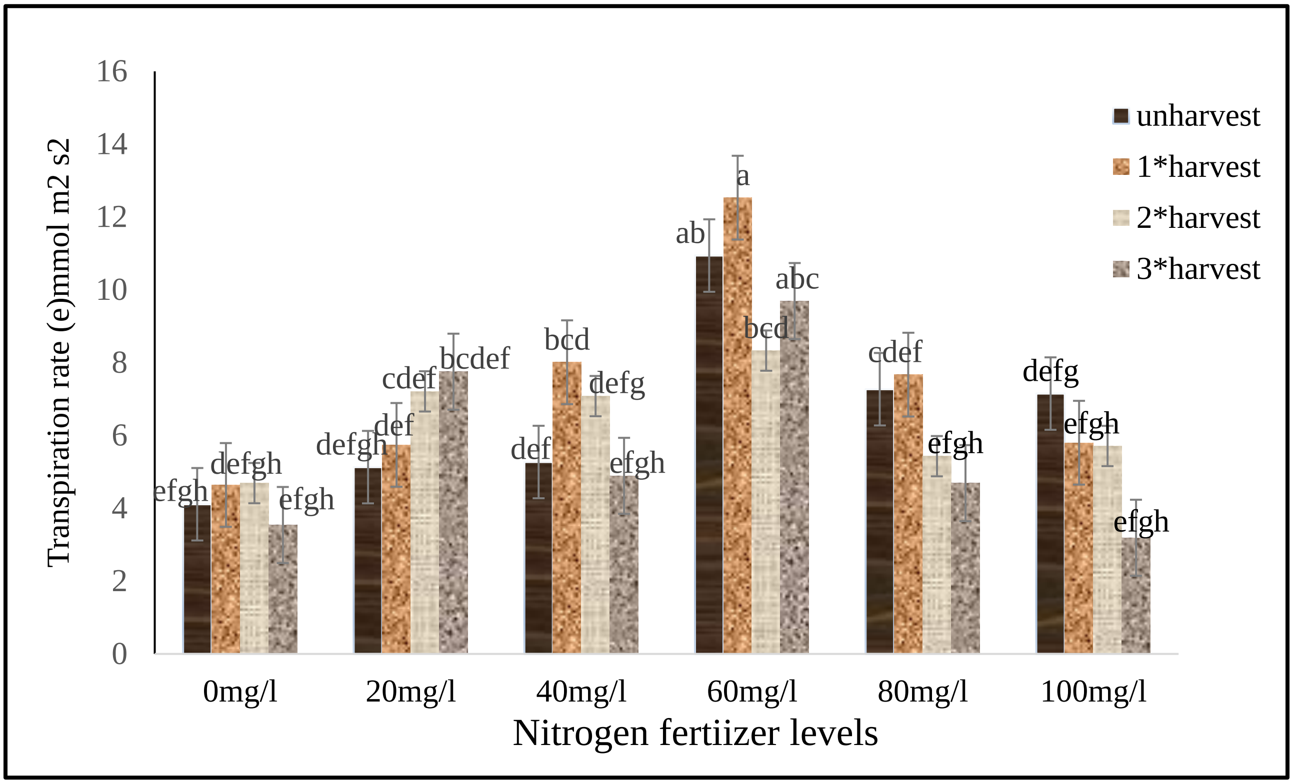

Figure 2. Interaction effect of nitrogen fertilizer and harvesting frequency on transpiration rate. While, means with different letter on the figure are statistically significant at P-values < 0.05, LSD (0.05), = 3.71and CV (%) = 21.4

4.3.3. Stomata Conductance ($molm^{-2}s^{-1}$)

Analysis of variance results revealed that stomata conductance was significantly ($P< 0.01$) influenced by the main effects of nitrogen fertilizer and harvesting frequency, but the interaction effect of nitrogen fertilizer and harvesting frequency had no significant ($p>0.05$) influence Stomata conductance of water hyacinth(Appendix table V).

Stomatal conductance is a major regulator of water vapor and carbon dioxide exchange between the leaf and the surrounding air, which directly affects plant growth. In this study, results indicate that as harvesting frequency increased, stomatal conductance of water hyacinth also significantly decreased, and the reduction was stronger when each treatment was harvested three times as compared to the other treatment. This result is related to leaf area and leaf number reduction, and as we know stomatal conductance has a direct relation with leaf morphology, in the previous discussion, as harvesting increases leaf number as well as leaf area reduced, this redaction affects the stomatal conductance of water hyacinth, and also it may be due to the harvesting stress, as harvesting increase, stomata conductance also decrease. This is the mechanism to face the stress by closing stomata, consequently to reduce photosynthesis and transpiration rate. This reduction may be due to stomata closure and may be due to higher temperature and higher VPD during the growth period (Table 2) (Urban et al., 2017). Increases in stomatal conductance, which regulates gas exchange (CO_2 and water), can allow plants to increase their CO_2 uptake and subsequently enhance transpiration and photosynthesis (Lawson et al., 2010).

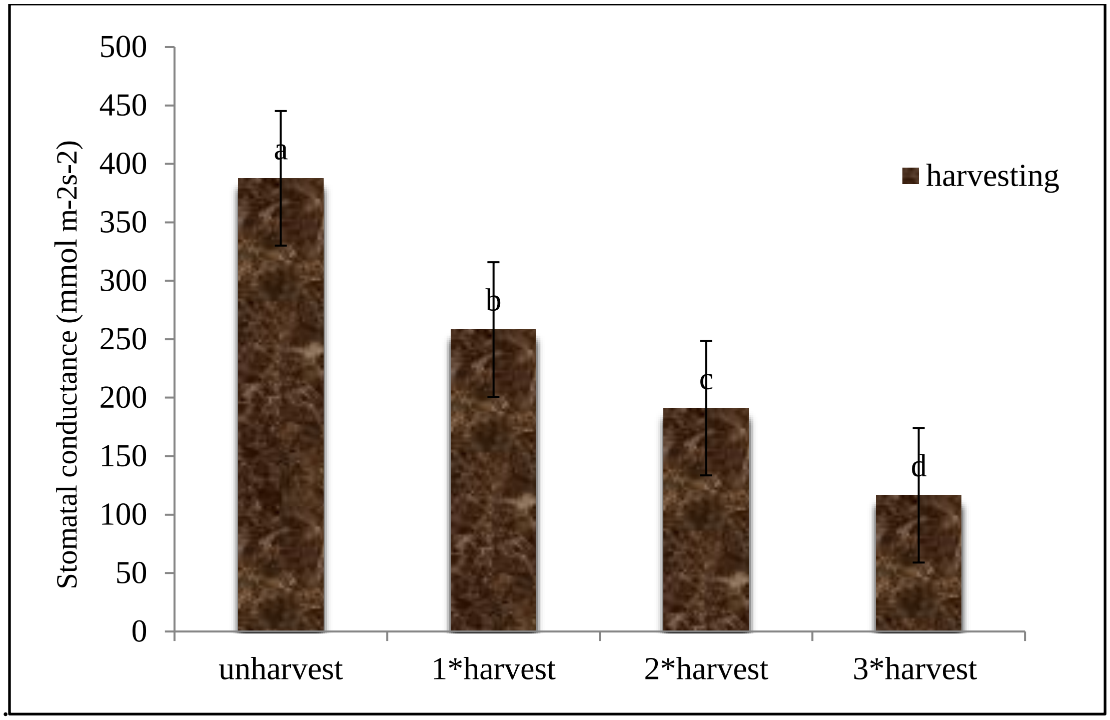

Figure3. Effect of harvesting frequency on stomatal conductance of water hyacinth plant. While, means with different letter on the figure are statistically significant at P-values < 0.05, LSD (0.05), = 45.43 and CV (%) = 28.40

The maximum stomatal conductance was observed with 60 mg/l N treatment, and the minimum stomatal conductance was observed with 0 mg/l N (figure 4). Nitrogen deficiency significantly reduced leaf area, leaf number, leaf chlorophyll content, and photosynthesis. Due to this nitrogen deficiency, it was mainly associated with lower stomatal conductance (Zhao et al., 2005). And also from our experiment, we can see that nitrogen concentrations above 60 mg/l do not influence the growth of water hyacinth in shoot growth; due to this, we observe the redaction of stomatal conductance. (Figure 4)

Stomatal conductance has highly related to photosynthesis and transpiration rate. Stomatal conductance is a function of the density, size and degree of opening of the stomata; with more open stomata allowing greater conductance, and consequently indicating that photosynthesis and transpiration rates are potentially higher (Pietragalla and Pask, 2012). Our finding also show their relationship, stomatal conductance decrease the photosynthesis and transpiration rate also decrease. The stomatal conductance reduction seems to be the main determinant for decreased photosynthesis and transpiration in this study. This could be a major cause of harvesting stress and nitrogen deficiency that induced decreases in CO2 assimilation capacity which results in decline of the growth as well as regeneration (Pagter et al., 2005).

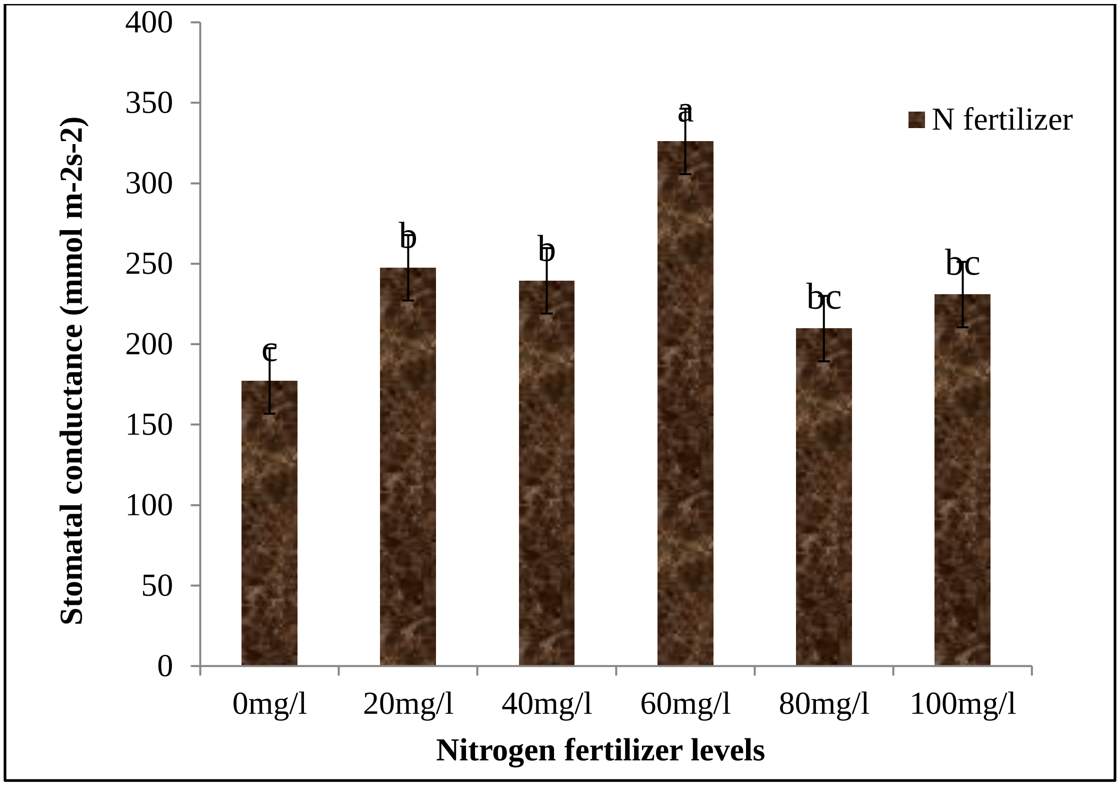

Figure 4. Effect of nitrogen fertilizer on stomatal conductance of water hyacinth plant. While, means with different letter on the figure are statistically significant at P-values < 0.05, LSD (0.05), = 55.64 and CV (%) = 28.40

4.3.4. Instantaneous Water Use Efficiency (IWUE)

Analysis of variance results revealed that instantaneous water use efficiency was significantly ($P<0.01$) influenced by the main effects of nitrogen fertilizer and harvesting frequency as well as the interaction effect (Appendix table V).

IWUE is s defined as the amount of carbon assimilated as biomass or grain produced per unit of water used by the crop. The maximum IWUE was observed from unharvested treated with 0 mg/l N (2.49) and the lowest observed from harvested thrice treated with 60 mg/l N (0.37) (Figure 5). This means the production of biomass is low in 0mg/l N treatment and the water used by the crop was mostly for transpiration. From our finding IWUE doesn't related to nitrogen fertilizer level but when we see harvesting frequency the highest IWUE was observed from control (unharvested) treatment and the lowest IWUE was observed from 3 time's harvested treatment. This implies as harvesting stress the water hyacinth have less leaf number and leaf area due to this it used the water effectively for the production of biomass. An increase in the water use efficiency was correlated with stomatal conductance redaction, which indicates that stomatal closure contributed to optimizing water use efficiency in plants under stress. As we see the concentration of nitrogen level, the highest IWUE was observed from 0mg/l (control) treatment and as the concentration of nitrogen increased the IWUE reduced significantly. This may be due to root morphology, in low nitrogen concentration the water hyacinth root was higher, and high absorption of water where there is no shortage of water in the treatment, this things may have indirect effect on water use efficiency of water hyacinth plant.

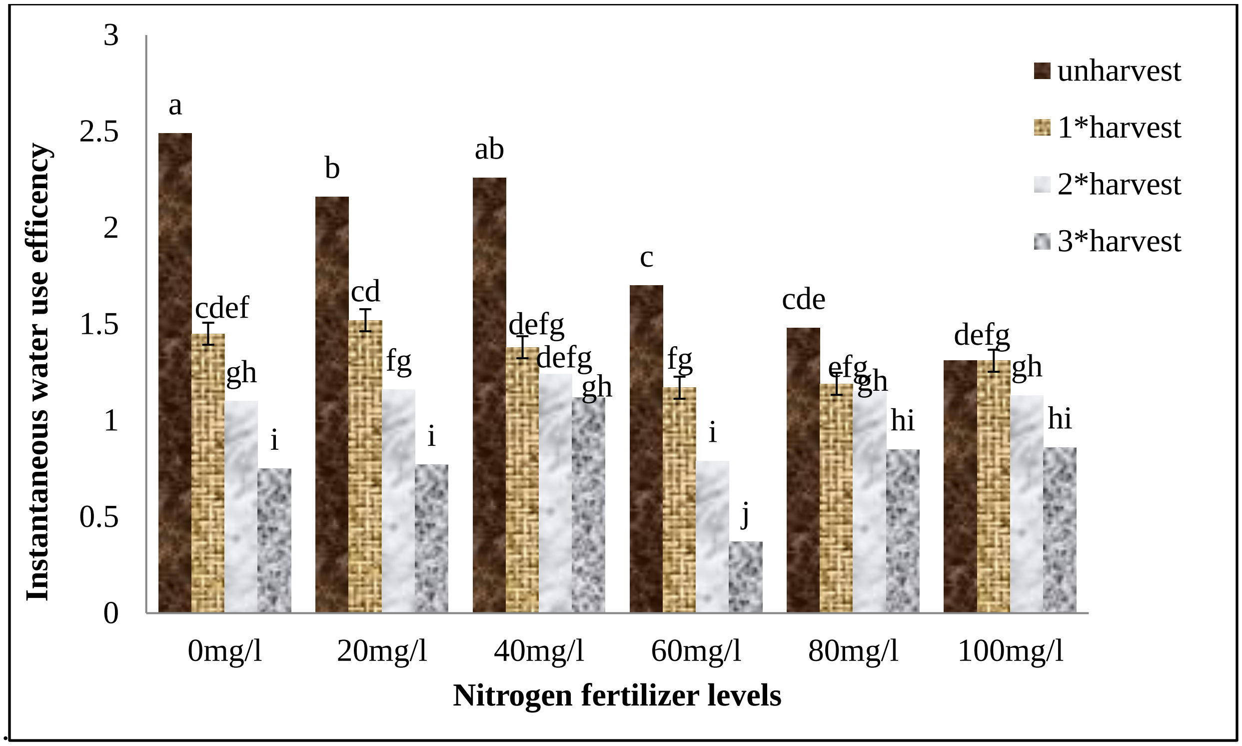

Figure 5. Interaction effect of nitrogen fertilizer and harvesting frequency on Instantaneous Water use efficiency (IWUE) of water hyacinth plant. While, means with different letter on the figure are statistically significant at P-values < 0.05, LSD (0.05), = 0.29 and CV (%) = 14.20

4.3.5. Relative Leaf Water Content (RLWC %)

Relative leaf water content is an important indicator of water status in plants; it reflects the balance between water supply to the leaf tissue and transpiration rate (Soltys-Kalina et al., 2016). Relative leaf water content of water hyacinth was significantly ($P < 0.001$) affected by the main factors of harvesting frequency but there was no significant ($P > 0.05$) influence by the main factors of nitrogen fertilizer level and the interaction effect (Appendix table V).

The results indicate that the highest RLWC was observed from control treatment. When harvesting interval increases, there is significant redaction on the RLWC of water hyacinth plants. This is due to harvesting increasing, leaf area and transpiration rate decrease. The redaction in leaf area and transpiration rate also have a direct effect on the water content of the leaf. (Kevyn and Catherine, 2012).

The results indicate that the highest relative leaf water content was found from the unharvest treatment (81.28%), and the lower result was observed from the twice and thrice times harvested treatments (72.14% and 70.13%), respectively (Figure 6). Decrease in leaf water potential, stomatal closure, decrease in internal CO2 concentration, all of which can lead to impairment of photosynthetic activity. Furthermore, Reducing water loss through stomatal closure, rolling or abscission of the leaf, and increased plant nutrient and water up-take through enhanced root development are mechanisms playing a role in maintaining the leaf water status (Singh and Reddy, 2011).

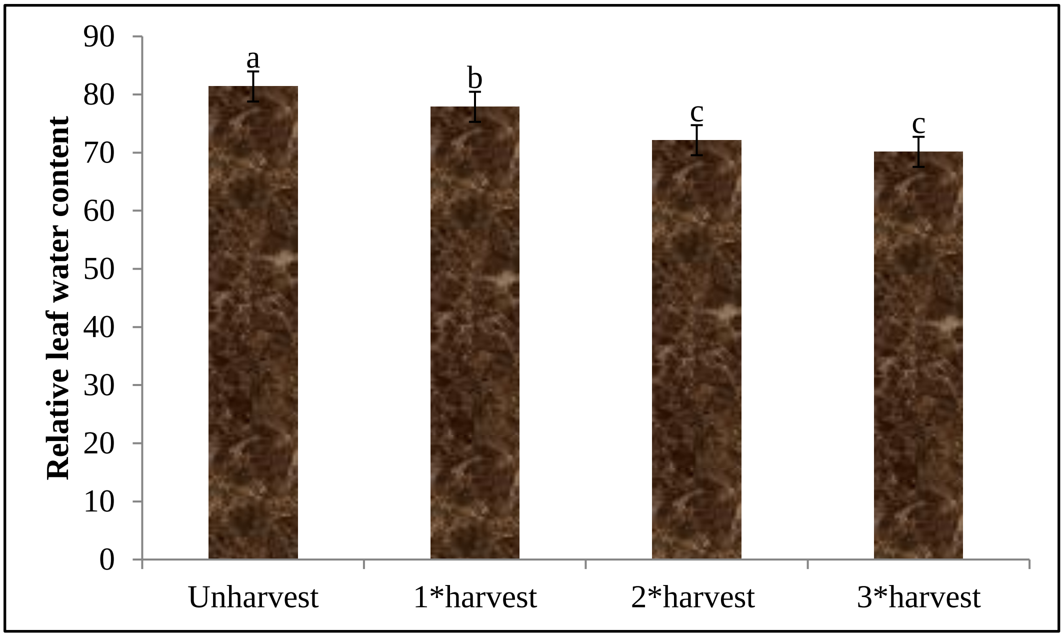

Figure 6. Effect of harvesting frequency on Relative leaf water content (RLWC %) of water hyacinth plant. While, means with different letter on the figure are statistically significant at P-values < 0.05, LSD (0.05), = 3.38 and CV (%) = 6.69

4.4. Response of Chlorophylls Concentration (μg ml^{-1}) of Water Hyacinth to Nitrogen Fertilizer and Harvesting Frequency

The result of analysis of variance revealed that leaf chlorophyll concentration (Chl a, Chl b, and total Chl.) were significantly ($p<0.01$) affected by nitrogen fertilizer but there was not significantly ($P \geq 0.05$) influence by harvesting frequency and the interaction effect (Appendix Table VI).

The highest mean chl a, chl b, and total Chl were observed from 60 mg/l nitrogen fertilizer treatments in all harvesting frequency. The lowest mean of Chl a, Chl b, and total Chl was observed from 0 mg/l nitrogen fertilizer treated with twice and thrice harvested.

This result indicated that nitrogen fertilizer and chlorophyll content of water hyacinth leaf is highly related, increasing nitrogen fertilizer up to 60 mg/l the chlorophyll concentration also increased, when we see the 80 mg/l and 100 mg/l nitrogen fertilizer treatment, the chlorophyll concentration was reduced. This means excess nitrogen have no effect on the concentration of chlorophyll a, b and total chlorophyll may be it also related with the alga bloom appear in the treatment 80 mg/l and 100 mg/l nitrogen fertilizer (Bao et al., 2015).

The reduction in chlorophyll concentration may be due to the relationship between nitrogen and chlorophyll. Chlorophyll is associated with nitrogen level, the increase of nitrogen application can increase the chlorophyll content (Walker et al., 2018). Nitrogen is also the primary building block for plant protoplasm. Protoplasm is the translucent substance that is the living matter in cells. This indicates that nitrogen deficiency affects the chlorophyll content of leaves. (Chandra et al., 2022).

The analysis of variance also showed that there is no relation between harvesting frequency and chlorophyll concentration of water hyacinth ($P \geq 0.05$). This means harvesting frequency doesn't have effect on the chlorophyll concentration of water hyacinth leaf and on its greens color, also this imply that stress due to harvesting have no effect on the chlorophyll concentration of water hyacinth plant grown in the pot.

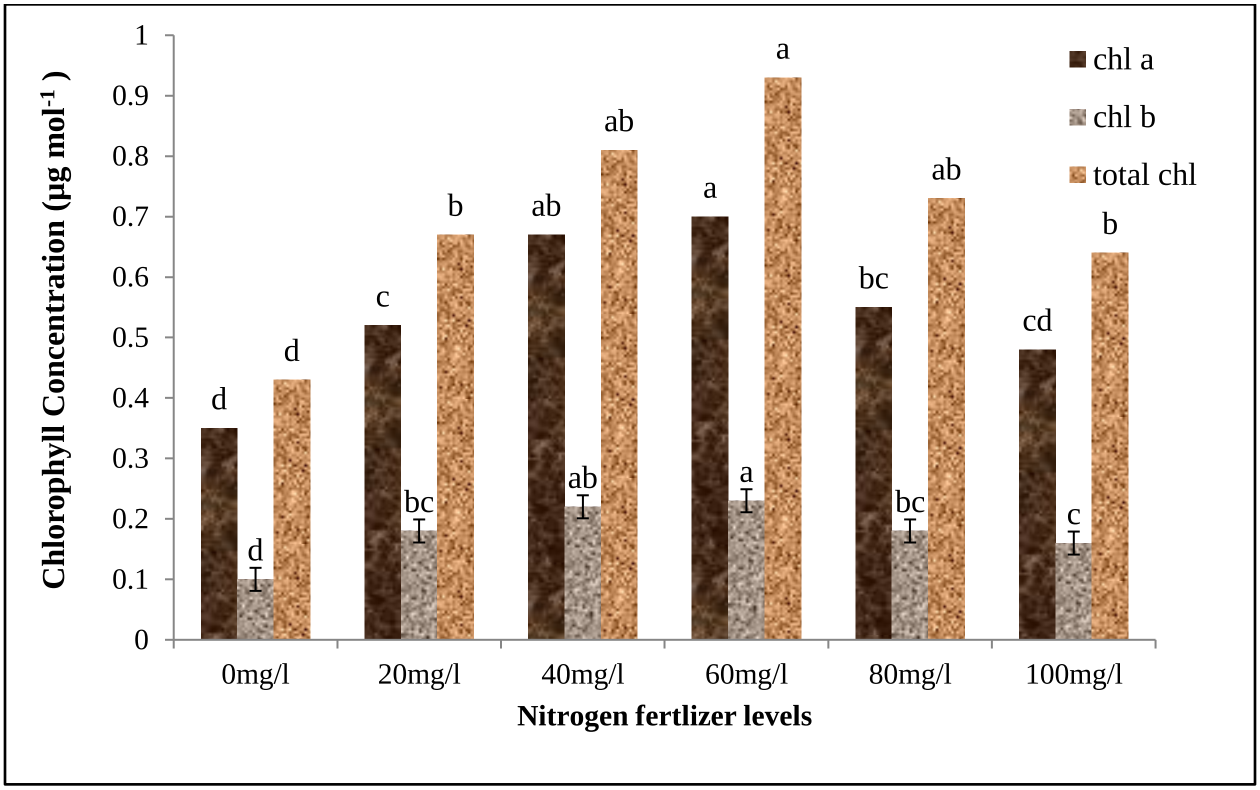

Figure 7. Effect of nitrogen fertilizer on chl a, chl, b and total chl concentration of water hyacinth plant. While, means with different letter on the figure are statistically significant at p- value < 0.05, for chl a. LSD (0.05) = 0.06 and CV (%) =10.25. for chl b. LSD (0.05) =0.08 and CV (%) = 12.65. For total chl. LSD (0.05) = 0.12 and CV (%) = 15.06

Where, chl a=concentration of chlorophyll a, ch b= concentration of chlorophyll b, total chl = concentration of total chlorophyll.

4.5. Fresh and Dry Biomass of Water Hyacinth

4.5.1 Water Hyacinth Shoot Fresh Weight, Root Fresh Weight, and Total fresh weight

The analysis of variance results showed, the interaction effect of harvesting frequency and nitrogen fertilizer significantly ($p < 0.05$) influenced the shoot fresh weight (Appendix Table I).

The higher value of shoot fresh weight was observed from 60mg/l N treated with un harvested, and the minimum value of shoot fresh weight was observed from 0, 80 and 100 mg/l N treated with thrice harvested. The results also revealed that the fresh weight of the shoot decreased with increasing harvesting frequency and nitrogen level. Our finding was agreement with the result of Gao et al. (2015), which stated that fresh water hyacinth experiments performed in the greenhouse showed that there was a significant increase in the production of fresh biomass at high N (N = 62.5 mg l^{-1}) concentrations.

The highest root fresh weight while observed from 0mg/l N treated with unharvest and lowest root fresh weight was observed from 0, 20, 80 and 100mg/l N treated with thrice harvested (Table 9). The results showed that both nitrogen fertilizer and harvesting frequency have an effect on the fresh weight of shoot and root of water hyacinth. As previously discussed, nitrogen fertilizer has a direct effect on leaf number, leaf area, and shoot growth, and the sum of those results was shown in the fresh wet shoot. Nitrogen is the primary nutrient needed for the growth and also gives the energy to speedy shoot growth (Fageria and Baligar, 2005). And also, harvesting frequency had a visible effect on the fresh wet of the shoot and roots, as we see from the results in all treatments; the fresh wet of the shoot and root decreased as harvesting frequency increased, but a high reduction in root fresh wet was observed on the 0 mg/l N treatment. This result indicate that as harvesting increases the water hyacinth plants became more stressed and removes its fibrous roots, making the plant weak (Madsen et al ., 1993). This also affects the regeneration potential of water hyacinth because the soluble carbohydrates stored in the roots and this stored carbohydrate give the plant the ability to regrowth after harvesting (Madsen et al., 1993).

Table 9. The Interaction Effect of Nitrogen Fertilizer and Harvesting Frequency on Fresh wet of Shoot and Root of Water Hyacinth.

Harvesting	**Nitrogen fertilizer (mg L^{-1})**					
	0	**20**	**40**	**60**	**80**	**100**
Shoot Fresh weight (g)						
unharvested	47.27^{e}	56.86^{cd}	65.52^{ab}	66.83^{a}	59.05^{bcd}	55.16^{d}
Harvested once	35.44^{f}	58.85^{bcd}	62.71^{abc}	65.18^{ab}	52.68^{de}	52.15^{df}
Harvested twice	22.43^{h}	24.83^{gh}	31.23^{fg}	34.03^{f}	23.16^{h}	22.00^{h}
Harvested thrice	10.33^{i}	13.26^{i}	24.46^{gh}	24.53^{gh}	13.73^{i}	9.50^{i}
Mean		38.8				
LSD		7.07				
CV%		11.10				
Root Fresh weight (g)						
unharvested	170.01^{a}	157.87^{ab}	112.37^{d}	113.37^{d}	154.16^{b}	109.65^{d}
Harvested once	133.67^{c}	126.17^{c}	107.47^{d}	109.87^{d}	108.63^{d}	107.80^{d}
Harvested twice	84.17^{g}	93.90^{ef}	94.13^{ef}	102.08^{df}	91.39^{ef}	91.43^{ef}
Harvested thrice	54.93^{i}	75.08^{gh}	86.71^{fg}	89.53^{f}	63.33^{hi}	62.63^{i}
Mean		104.18				
LSD		12.24				
CV%		7.15				

Where, LSD and CV indicate least significance difference and Coefficient of variation, respectively. Means in the same column followed by the same letter are not significantly different at the 5% probability level

Total fresh weight of water hyacinth was also significantly ($p < 0.001$) affected by nitrogen fertilizer level, harvesting frequency, the interaction effect (Appendix IV). Under all nitrogen levels, the control (un harvested) treatment had the highest total fresh weight while the three-times harvesting treatment resulted in the lowest total biomass. The highest total fresh weight was obtained from 0 mg/l and 20 mg/l N treated with control (un harvested), while the lowest were obtained from 0 mg/l and 100 mg/l N treated with thrice harvested (Table 10). This indicates that from total fresh weight the highest value is from root and as harvesting increase their was high reduction in total fresh weight of water hyacinth.

Table 10. The Interaction Effect of Nitrogen Fertilizer and Harvesting Frequency on Total Fresh Weight of Water Hyacinth

	Nitrogen fertilizer (mg L^{-1})					
Harvesting	**0**	**20**	**40**	**60**	**80**	**100**
Total fresh weight (g)						
Unharvested	217.28^{a}	214.73^{a}	179.20^{bc}	178.89^{bc}	213.22^{a}	169.11^{cde}
Harvested once	185.02^{b}	164.82^{de}	170.18^{cde}	175.05^{bcd}	161.32^{e}	159.95^{e}
Harvested twice	106.60^{h}	118.73^{gh}	125.37^{fg}	136.11^{f}	114.55^{gh}	113.43^{gh}
Harvested thrice	65.27^{j}	88.35^{i}	111.18^{h}	114.07^{gh}	77.07^{ij}	72.13^{j}
Mean		142.98				
LSD		13.32				
CV %		5.67				

Where, LSD and CV indicate least significance difference and Coefficient of variation, respectively. Means in the same column followed by the same letter are not significantly different at the 5% probability level.

4.5.2. Water Hyacinth Shoot Dry Weight, Root Dry Weight, and Total Dry Weight

Harvesting frequency, nitrogen fertilizer, and their interaction were significantly ($P \leq 0.001$) affect the dry weight of water hyacinth (Appendix III). Our finding indicate that the control treatment showed a higher value in all nitrogen level and the minimum value was observed from three times harvest in all nitrogen treatment. The result also showed that the dry weight of water hyacinth shoot was related to nitrogen fertilizer. A higher value was observed at 40 mg/l and 60 mg/l combined with control (un harvest) treatment, and the minimum value was observed at 0 mg/l and 100 mg/l combined with thrice harvested (Table 11). The 0 mg/l N treated with unharvested produced good results for the root dry weight. Thus, minimum dry weight of the shoot of water hyacinth was obtain from 100mg/l nitrogen treated with thrice harvested treatment (Table 11). The carbohydrate reserve in root are believed to play an important role in resprouting plant (Bullock et al., 2011). And also natural regeneration is a key component for securing the sustainability of perennial plants. Harvesting frequency affect the reserve carbohydrate and the capacity of water hyacinth to regrowth.

Table 11. The interaction Effect of Nitrogen Fertilizer and Harvesting frequency on Dry Wet of Shoot and Dry Wet of Root of Water Hyacinth.

Harvesting	Nitrogen fertilizer (mg L^{-1})					
	0	**20**	**40**	**60**	**80**	**100**
Dry wet of shoot (g)						
unharvested	14.86^{c}	16.80^{b}	18.43^{a}	19.33^{a}	14.83^{c}	12.23^{de}
Harvested once	10.06^{g}	11.30^{efg}	18.10^{ab}	18.36^{a}	11.05^{efg}	13.56^{cd}
Harvested twice	7.10^{i}	10.40^{fg}	11.50^{ef}	11.66^{ef}	8.63^{h}	8.56^{h}
Harvested thrice	4.53^{k}	7.40^{hi}	6.43^{ij}	6.96^{i}	5.50^{jk}	4.46^{k}
Mean		11.33				
LSD		1.38				
CV%		7.43				
Dry wet of root (g)						
Unharvested	24.03^{a}	22.40^{b}	16.16^{e}	15.90^{ef}	20.26^{c}	21.30^{bc}
Harvested once	17.56^{d}	18.46^{d}	14.80^{fg}	13.90^{gh}	12.80^{hi}	12.56^{i}
Harvested twice	11.53^{ijk}	12.66^{hi}	11.80^{ij}	12.23^{i}	10.76^{jkl}	9.36^{m}
Harvested thrice	9.96^{lm}	10.03^{lm}	10.30^{klm}	10.53^{jklm}	8.03^{n}	6.90^{n}
Mean		13.92				
LSD		1.27				
CV%		5.58				

Where, LSD and CV indicate least significance difference and Coefficient of variation, respectively. Means in the same column followed by the same letter are not significantly different at the 5% probability level.

Water hyacinth total dry mass was also highly significantly ($p \leq 0.001$) influenced by the main effect and the interaction effect of harvesting frequency and nitrogen fertilizer (Appendix IV). The highest total dry weight of water hyacinth was observed at 0 mg/l and 20 mg/l N treated with unharvested treatment. This result was due to the high value of the root dry weight of water hyacinth. The minimum total dry weight of water hyacinth was observed from thrice harvest with 100 mg/l nitrogen treatment. Depending on our results, when harvesting frequency increases, the dry matter of water hyacinth decreases (Table 12).

Table 12. The Interaction Effect of Nitrogen Fertilizer and Harvesting Frequency on Total Dry Mass of Water Hyacinth.

Harvesting frequency	**Nitrogen fertilizer (mg L^{-1})**					
	0	**20**	**40**	**60**	**80**	**100**
Total dry mass (g)						
Unharvested	38.89[a]	37.26[a]	34.60[bc]	35.23[b]	35.10[b]	33.53[c]
Harvested once	27.63[f]	29.76[e]	32.90[cd]	32.26[cd]	23.85[g]	26.13[f]
Harvested twice	18.63[hi]	23.06[g]	23.30[g]	23.90[g]	19.40[h]	17.93[hi]
Harvested thrice	14.50 [J]	17.43[hi]	16.73[i]	17.50[hi]	13.53[j]	11.36[k]
Mean		25.26				
LSD		2.01				
CV %		4.85				

Where, LSD and CV indicate least significance difference and Coefficient of variation, respectively. Means in the same column followed by the same letter are not significantly different at the 5% probability level.

4.6. Correlation Analysis

4.6.1. The Correlation Between Morphological, Physiological, and Dry Matter Components Exposed to Various Nitrogen Levels and Harvesting Frequencies.

The correlation values explained the association between different analyzed parameters and clearly indicated the magnitude of their relationships. Correlation analysis indicated that all morphological traits had significant ($P<0.001$) and positive correlation with physiological traits except transpiration rate (E) and instantaneous water use efficiency (IWUE) (Table 13). Transpiration rate had a negative ($P >0.001$) correlation with petiole diameter and root length, and IWUE. Leaf number and Leaf area showed a positive and strong correlation with leaf number ($r = 0.93$ and $r = 0.90$) (Table 13).

Consequently, plant height, leaf number, and leaf area of water hyacinth have been strongly related to physiological traits. Since morphological traits play a crucial role in physiological traits,

The correlation result also demonstrates a correlation between physiological traits and the dry matter component. All physiological traits had a significant ($P<0.001$) and positive moderate correlation with the dry matter component (Table 14). Shoot dry weight (SDW) with photosynthesis rate (A) had a strong positive correlation ($r= 82$), and the chlorophyll concentration a,b and a+b had a low positive correlation with dry matter component (Table 14).

Table 13; Pearson Correlation coefficient(r) among morphological traits, physiological traits and dry mater component of water hyacinth grown under different nitrogen levels and harvesting frequency

	PH	LN	LA	LT	NS	PD	RL	RD	RN
A	0.81	0.81	0.87	0.58	0.75	0.36	0.14	0.60	0.70
Gs	0.70	0.77	0.80	0.10	0.64	0.50	0.09	0.59	0.66
E	0.37	0.40	0.40	0.80	0.39	-0.04	-0.09	0.23	0.24
IWUE	0.51	0.57	0.60	0.64	0.47	0.54	0.35	0.56	0.67
Ch a	0.53	0.44	0.46	0.06	0.43	0.44	-0.28	0.07	0.19
Ch b	0.53	0.42	0.46	0.09	0.39	-0.09	-0.27	0.05	0.16
Chl a+b	0.45	0.41	0.42	0.04	0.37	-0.02	-0.28	0.07	0.16
SFW	0.77	0.93	0.90	0.55	0.82	0.62	0.13	0.70	0.83
RFW	0.52	0.67	0.65	0.70	0.53	0.70	0.44	0.85	0.86
TFW	0.67	0.85	0.81	0.69	0.70	0.71	0.34	0.86	0.92
SDW	0.81	0.90	0.91	0.52	0.84	0.51	0.08	0.65	0.77
RDW	0.46	0.66	0.65	0.76	0.45	0.75	0.60	0.87	0.88
TDM	0.70	0.86	0.86	0.71	0.71	0.70	0.38	0.84	0.92

Where, PH=Plant height, LN=Leaf number, LA=Leaf area, NS= Number of stolon, PD= Petiole diameter, RL=Root length, RD=Root diameters, RN=Root number, E=Transpiration rate, IWUE= Instantaneous water use efficiency, A=Photosynthesis rate, g_s= Stomatal conductance all values were significantly and positively correlated at ($p \leq 0.001$)

Table 14: Pearson Correlation coefficient(r) among physiological traits and dry matter component of water hyacinth grown under different nitrogen fertilizer level and harvesting frequency.

	E	**IWUE**	**A**	**g_s**	**Chl a**	**Chl b**	**Chl a+b**
FWS	0.37	0.59	0.76	0.73	0.42	0.39	0.41
FWR	0.22	0.60	0.63	0.52	0.13	0.12	0.14
TBM	0.31	0.65	0.74	0.66	0.27	0.25	0.27
SDW	0.34	0.57	0.82	0.78	0.50	0.48	0.46
RDW	0.19	0.60	0.61	0.57	0.10	0.11	0.10
TDM	0.29	0.65	0.79	0.75	0.33	0.33	0.31

Where, E=Transpiration rate, IWUE= Instantaneous water use efficiency, A=Photosynthesis rate, g_s= stomatal conductance, Chl a= chlorophyll a, Chl b= chlorophyll b, Chl a+b=total chlorophyll, FWS= fresh wet of shoot , FWR=fresh wet of root , TBM= Total bio mass, SDW= Shoot dry wet, RDW =Root dry wet and TDM= Total dry mass all values were significantly and positively correlated at ($p \leq 0.001$)

5. CONCLUSION AND RECOMMENDATION

Water hyacinth (*Eichhornia crassipes*) is a perennial, herbaceous, free-floating, and flowering invasive aquatic plant of the genus *Eichhornia* in the Pickerelweed family (*Pontederiaceae*). Water hyacinths have been translocate almost all over the world except Antarctica. Mainly distributed as ornamental plants, Due to its rapid growth and proliferation capacity, it became one of the top ten worst weeds. The plant can double itself within 14 days. In Ethiopia, the first reports on the water hyacinth were from Koka dam and the Awash River in 1956. Where it emerges from Koka Lake and then distributes to other bodies of water over time, know it became the most dangerous weed in Ethiopia water bodies.

The major factors for the invasion of water hyacinths in water bodies are their vegetative reproduction capacity, the swallow petiole, the root morphology and also the climate change, fertilizer input to the water bodies. Due to the problem related to the water hyacinth invasion, several research and study projects cited elsewhere in the world were conducted at different times and places. But there are no effective results or methods for the successful control of water hyacinths in the water bodies. Most of them effectively remove or kill the leaves and the shoot part but not effective on the roots. Due to its perennial nature, water hyacinth stores carbohydrates in its tissues, especially in its root. The stored carbohydrates can be mobilized at a later date or after harvesting and are used by the plant to provide energy and carbon for the survival and regeneration. This makes the water hyacinth difficult to control. For the management of water hyacinth, the best we can hope for is to reduce its biomass and reproductive potential, and to reduce the surface area covered by the weed need current knowledge is essential.

Our experiment was conducted under a shade house condition from March to June 2021 at Hawassa University College of Agriculture. The treatment was comprised of a factorial combination of four harvesting frequencies (unharvested, harvested once, harvested twice, and harvested thrice) and six nitrogen fertilizer levels (0, 20, 40, 60, 80, and 100 mg/l) with three replications. A complete randomized design (CRD) was employed for the study.

The result of the study shows that morphological and growth parameters have been significantly affected by the interaction effect of nitrogen fertilizer and harvesting frequency. The maximum result was observed from 60 mg/l N treated with unharvested, but in root growth, the maximum result was observed from 0 and 20 mg/l N treated with unharvested. The newborn stolon was high in all one time harvested treatments at all nitrogen levels. The swallow petiole was observed from 0 and 100 mg/l N treated with unharvested.

And also, the result shows that the physiological parameters, photosynthesis, transpiration, and IWUE are significantly affected by the interaction effects of nitrogen fertilizer and harvesting frequency, Stomatal conductance was affected by the main effect, but the interaction effect has not affected the stomata conductance. Relative leaf water content (RLWC) is only affected by harvesting frequency, harvesting frequency increases, RLWC decreases. The chlorophyll concentration of water hyacinth only affects by nitrogen fertilizer level, the highest chlorophyll concentration was observed from a 60 mg/l N treatment.

The 60 mg/l N treated with unharvested had the highest results in fresh and dry weight of the shoot, and for root fresh weight and dry weight, the 0 mg/l N treated with unharvested had the highest value.

In conclusion, the finding suggests that nitrogen fertilizer level and harvesting frequency have a determinate effect on the morphology, physiology, and dry matter composition of water hyacinth. The reduction in photosynthesis induced by nitrogen fertilizer up to 60mg/l and harvesting frequency as a consequence of leaf morphology, stomatal conductance, and chlorophyll content results in a significant reduction in the dry matter of water hyacinth.

From this study, 60 mg/l and 40 mg/l nitrogen fertilizer levels have a good tolerance capacity for harvesting frequency compared to 0 mg/l, 20 mg/l, 80 mg/l, and 100 mg/l nitrogen fertilizer levels. It becomes more challenging to control the water hyacinth invasion if we can't control the level of nutrient dumped in to water ways from industrial and fertilizer run-off.

The simplest assessment of tolerance in response to harvesting frequency is the capacity of the plant to grow and regenerate (regrowth) after harvesting. This result also suggests that a good nitrogen level (40mg/l and 60mg/l) in the water with a determinate growth habit may have the potential to survive the harvesting stress.

It is possible to interpret the traits associated with harvesting tolerance as being due to its unique genetic viability and morphological adaptation characteristics, especially on its swallow petiole, stolon production and on its root morphology plasticity under high and low nitrogen fertilizer levels and harvesting frequency.

To properly manage or control water hyacinth, it must be removed more quickly than it reproduction rate. Most of our water bodies have nitrogen concentrations below 20 mg/l. Our countries is currently fighting to control the spread of water hyacinth, know it is the critical time to make strategy considering the regeneration potential of water hyacinth on lakes and rivers by frequently harvesting other ways it became more dangerous and complicated as the nutrient level in water increase.

Recommendation

Based on the present findings, some recommendations are stated.

- The growth rate of water hyacinths (*Eichhornia crassipes*) is faster, particularly in terms of shoot growth, root growth, and stolon production. As a result, it is advised to harvest frequently to prevent its invasion. Only one time's harvesting causes the water hyacinth to use the stored carbohydrate to produce more stolons quickly and abundantly and it cover the area quickly and is became more dangerous and it is also risk for management practice. Due to this the paper recommend frequent harvesting is a must as we take harvesting as a control method.
- Water hyacinth is a perennial herbaceous plant. This nature makes the plant difficult to control; hence, it is recommended to harvest two to three times before the leaves start photosynthesis at a 15-day interval in order to weaken the plant.
- The research suggests that a nitrogen concentration below 40 mg/l is ideal for management because it is one of the most manageable techniques to control the spread of water hyacinth in the water bodies are control or reduce the fertilizer and pollutants that enter water bodies.
- The most significant limitation in water hyacinth management was a lack of knowledge or limited concern about root morphological character and its regeneration potential, which must be addressed for effective management practices that use integrated weed management practices to make the weeds weak, in their carbohydrate accumulation, and make it suitable for the other control practice its need systematic and scheduled harvesting program, which is a must. This paper recommends two to three times harvests to degenerate the regrowth potential of water hyacinth.

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7. APPENDIX

Appendix Table II. Analysis of variance for plant height (cm), petiole diameter (cm), number of stolon and leaf number of water hyacinth treated with different nitrogen fertilizer under harvesting frequency.

Source variation	DF	Parameters			
		Plant height	Petiole diameter	Number of scar	Leaf number
N. fertilizer (N)	5	317.48***	45.67***	2.59***	27.35***
Harvesting (H)	3	593.50***	204.73***	9.85***	219.38***
N x H	15	29.51***	3.08***	0.19**	2.09***
Error	46	5.19	0.24	0.07	0.19
CV	71	11.26	5.83	14.18	6.29

*Where, ** and *** indicates significant difference at P≤ 5% and P≤ 1% probability levels, respectively. df = degree of freedom, CV= coefficient of variance, N *H=interaction among nitrogen fertilizer and harvesting frequency.*

Appendix Table III. Analyses of variance for leaf area (cm^2), leaf thickness (cm) ,root diameter(cm), root number and root length(cm) of water hyacinth treated with different nitrogen fertilizer under harvesting frequency.

Source variation	DF	Parameters				
		Leaf area	Leaf thickness	Root diameter	Root number	Root length
N. fertilizer (N)	5	76846***	0.13***	27.42***	19.85**	322.12***
Harvesting (H)	3	388313***	0.17***	565.29***	4052.64***	78.71***
N x H	15	4603***	0.005***	25.3***	72.40***	8.19***
Error	46	873	0.00073	1.025	3.99	1.62
CV	71	9.75	5.07	4.69	4.30	5.19

*Where,** and *** indicates significant difference at P≤ 5% and P≤ 1% probability levels, respectively. df = degree of freedom, CV= coefficient of variance, N *H=interaction among nitrogen fertilizer and harvesting frequency.*

Appendix Table IV. Analyses of variance for fresh wet of shoot (g), fresh wet of root (g),total bio mass(g), dry wet of shoot (g), dry wet of root (g) and total dry mass (g) of water hyacinth treated with different nitrogen fertilizer under harvesting frequency

Source variation	DF	Parameters			
		Fresh wet of shoot(g)	Fresh wet of root (g)	Dry wet of shoot (g)	Dry wet of root (g)
N. fertilizer (N)	5	593.23***	645.1***	55.23***	26.86***
Harvesting (H)	3	7863.76***	13917.7***	365.52***	396.47***
N x H	15	40.77*	891.1***	6.80***	11.80***
Error	46	18.55	55.5	0.71	0.6
CV	71	11.10	7.15	7.43	5.58

*Where,** and *** indicates significant difference at P≤ 5% and P≤ 1% probability levels, respectively. df = degree of freedom, CV= coefficient of variance, N *H=interaction among nitrogen fertilizer and harvesting frequency.*

Appendix Table V. Analyses of variance for total bio mass (g), root (g) and total dry mass (g) of water hyacinth treated with different nitrogen fertilizer under harvesting frequency.

Source variation	DF	Parameters	
		Total bio mass (g)	Total dry wet (g)
N. fertilizer (N)	5	969.7***	45.34***
Harvesting (H)	3	42000.2***	1494.58***
N x H	15	818.5***	12.53***
Error	46	65.8	1.50
CV	71	5.67	4.85

*Where,** and *** indicates significant difference at P≤ 5% and P≤ 1% probability levels, respectively. df = degree of freedom, CV= coefficient of variance, N*H=interaction among nitrogen fertilizer and harvesting frequency.*

Appendix Table VI. Analysis of variance for photosynthesis efficiency (A) (µ mol m-2s-1), stomata conductance (g_s)(mmol m-2s-1), transpiration rate (E) (mmol m-^{2}s-^{1}) and, Instantaneous water use efficiency(IWUE) and relative leaf water content (RLWC)of water hyacinth treated with different nitrogen fertilizer under harvesting frequency.

Source variation	DF	Parameters				
		A	g_s	E	IWUE	RLWC
N. fertilizer (N)	5	41.71***	29736**	125.18***	0.47***	35.68ns
Harvesting (H)	3	128.97***	238611***	11.88ns	3.97***	345.19***
N x H	15	4.64*	7716ns	25.81***	0.16***	24.32ns
Error	46	1.87	4585	5.11	0.033	25.45
CV	71	16.59	28.40	31.45	14.20	6.63

*Where, ns, * and *** indicates non-significant difference at P≤ 5%, significant difference at P≤ 5% and P≤ 1% probability levels, respectively. df = degree of freedom, CV= coefficient of variance, N* H= interaction among nitrogen fertilizer and harvesting frequency*

Appendix Table VII. Analysis of variance for chlorophyll a (chl a), chlorophyll b (chl b), total chlorophyll (chl a+b) concentration of water hyacinth treated with different level of nitrogen fertilizer under different harvesting frequency

Source variation	DF	Parameters		
		Chl a	Chl b	TC
N. fertilizer (N)	5	0.193**	0.026***	0.337**
Harvesting (H)	3	0.065ns	0.0063ns	0.126ns
N x H	15	0.014ns	0.0015ns	0.037ns
Error	46	0.027	0.00359	0.061
CV	71	30.12	32.80	35.06

*Where, ns, * and *** indicates non-significant difference at P≤ 5%, significant difference at P≤ 5% and P≤ 1% probability levels, respectively. df = degree of freedom, CV= coefficient of variance, N* H= interaction among nitrogen fertilizer and harvesting frequency*

SKETCH OF BIOGRAPHY

The author, **Yedidya Biratu Deressa**, was born on June 13, 1994, GC in Hawassa, Sidama regional state, Ethiopia. In 2000 GC admitted to grades 1–4 of primary education at Hawasa Adventist primary school and grades 5–8 at Hawassa Tabor Mekan Eyasus primary school. She attended high school (9–10) and preparatory school (11–12) at Hawassa Tabor Evangelical Secondary and Preparatory School. After completing the university entrance examination, she joined Jimma University in 2012, to pursue her first degree and graduated with a BSc in plant science in July 2015. After her graduation, she worked at the Hawassa City Administration and Agriculture Department until now. In 2019 GC, she got a chance to attend a master's program and joined the Graduate Program in Horticulture at Hawassa University.