

Comparitive Studies on Phytochemical Screening, Total Phenolic, Total Flavoid Content and Metal Analysis of Hydroponic (Nft System) and Geoponic Cultivated Spinach

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Abstract

Today, the agriculture is the key development in the rise of sedentary human civilization, whereby farming of domesticated species create food surpluses that nurture the development of civilization.

For global food security, the agricultural sector of the world economy must achieve a production level that ensures adequate food supply to feed the increasing population as well as provides raw materials for the industries.

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For this reasons, now a day the crops are excessively exposed to chemical /synthetic fertilizers and pesticides for getting more yield within the limit of time for money. By consuming such food material, a large proportion of the population has been suffering from a lot of diseases and disorders because they contain arsenic, mercury, nitrates, and lead more than permitted levels.

In view of health hazards posed by indiscriminate use of chemical fertilizers and pesticides, extremely difficult situations of land and water managements and adverse influences of climatic conditions such as heavy rains, air pollution, water pollution, soil pollution, heavy sun effect) on the quality and quantity of agricultural products , we have panned the cultivation of spinach hydroponically. The objective of present study was to compare the phytochemical constituents of geoponic and hydroponic cultivated spinach and screening for antioxidant activity based on their phenolic and flavonoid contents .Further, how much extent the hydroponic cultivation is influencing the presence of metals by carrying out metal analysis by Inductively Coupled Plasma/Optical Emission Spectrometry.

We have observed that the plant, which was cultivated by hydroponics, have a good growth, and also shows a significant content of phenolic and flavonoid than the geoponics. The most important thing was that the plant (hydroponics) don't have heavy metals .but geoponics have both the Lead and Arsenic which are very dangerous diseases to the human life.

List of abbreviations: %: percentage; ml : millilitre; µg : micro gram; g : gram; kg: Kilogram; min: minute; sec: seconds; W/v: weight/volume; V/v: volume/volume; fig: figure; conc.: Concentrated; ppt: precipitated

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Introduction

Historically, humans secured food through plants and animal source. Food is any substance [1] consumed to provide nutritional support for an organism. Today, the agriculture is the key development in the rise of sedentary human civilization, whereby farming of domesticated species create food surpluses that nurture the development of civilization.

For global food security, the agricultural sector of the world economy must achieve a production level that ensures adequate food supply to feed the increasing population as well as provides raw materials for the industries.

For this reasons, now a day the crops are excessively exposed to chemical/synthetic fertilizers and pesticides for getting more yield within the limit of time for money. By consuming such food material, a large proportion of the population has been suffering from a lot of diseases and disorders because they contain Arsenic, Mercury, Nitrates, and Lead more than permitted levels.

Pesticides may cause acute and delayed health effects in people who are exposed [3]. Their exposure can cause a variety of adverse health effects, ranging from simple irritation of the skin and eyes to more severe effects such as affecting the nervous system, mimicking hormones causing reproductive problems, and causing cancer [4]. A 2007 systematic review found that “most studies on non-Hodgkin lymphoma and leukemia showed positive associations with pesticide exposure” and thus concluded that cosmetic use of pesticides should be decreased [5]. There is substantial evidence of associations between organophosphate insecticide exposures and neurobehavioral alterations [6-9]. Limited evidence also exists for other negative outcomes from pesticide exposure including neurological, birth defects, and fetal death. [10]

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Pesticide use raises a number of environmental concerns also. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non-target species, air, water and soil. [12] Pesticide drift occurs when pesticides suspended in the air as particles are carried by wind to other areas, potentially contaminating them. Pesticides are one of the causes of water pollution, and some pesticides are persistent organic pollutants and contribute to soil contamination.

The World Health Organization and the UN Environment Programme estimate that each year, 3 million workers in agriculture in the developing world experience severe poisoning from pesticides, about 18,000 of whom die. Owing to inadequate regulation and safety precautions, 99% of pesticide related deaths occur in developing countries that account for only 25% of pesticide usage.

In developing countries, there is no thorough control over the usage of pesticides. For example, more than 60 pesticides banned in other countries in use in India. To avoid this problem food safety and food security are monitored by agencies like the International Association for Food Protection, World Resources Institute, World Food. They address issues such as sustainability, biological diversity, climate change, nutritional economics, population growth, water supply, and access to food.)Programme, Food and Agriculture Organization, and International Food Information Council.

Some countries follow the International Maximum Residue Limits-Codex Alimentarius to define the residue limits; this was established by Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) in 1963 to develop international food standards, guidelines codes of practices, and recommendation for food safety.

Apart from these problems, the other major problems of cultivation are availability of vast land, water supply/scarcity, shortage of labour etc., to avoid indiscriminate usage of pesticides and chemical fertilizers, and water and land problem in conventional cultivation, we have thought of some alternative method of cultivation, which is viable and effective. Therefore, we have contemplated to select a technique called as hydroponics.

Hydroponics is a subset of hydroculture, the method of growing plants without soil, using mineral nutrient solutions in a water solvent. Terrestrial plants may be grown with only their roots exposed to the mineral solution, or the roots may be supported by an inert medium, such as perlite or gravel.

The cultivation of plants by using hydroponics techniques such as Wicks system, Water culture, Ebb and Flow system, Drip system, Nutrient Flim Technique, Aeroponics system. Substrates are also used for support the plant to stay in the hydroponics system. Some of the substrates used in hydroponics were following Grow Stones, Coir Peat, Rice Husks, Perlite, Vermiculite, Pumice, Sand, Gravel, Wood Fibre, Sheep Wool, Rock Wool, Brick Shards, Polystyrene Packing Peanuts Selection of the medium is an important parameter. Different media are appropriate for different growing techniques

Conditions to be maintained in hydroponics technique: All plants need the same basics to survive and grow healthily. In soilless gardening there are definite factors to consider achieving optimal hydroponic growing conditions. These are generally the same basics as with soil-based gardening. However, there are a few specifics of hydroponic growing conditions that all beginners should be aware of before getting started.

Six considerations for the optimum conditions of growth in hydroponics are Water, light, Nutrients, Temperature, oxygen, Structure/ Support:

List of nutrition required and its uses:

Mineral Elements Required by Plants		
ELEMENT	ABSORBED FORM	MAJOR FUNCTIONS
MACRONUTRIENTS		
Nitrogen (N)	NO_3^- and NH_4^+	In proteins, nucleic acids, etc.
Phosphorus (P)	H_2PO_4^- and HPO_4^{2-}	In nucleic acids, ATP, phospholipids, etc.
Potassium (K)	K^+	Enzyme activation; water balance; ion balance; stomatal opening
Sulfur (S)	SO_4^{2-}	In proteins and coenzymes
Calcium (Ca)	Ca^{2+}	Affects the cytoskeleton, membranes, and many enzymes; second messenger
Magnesium (Mg)	Mg^{2+}	In chlorophyll; required by many enzymes; stabilizes ribosomes
MICRONUTRIENTS		
Iron (Fe)	Fe^{2+} and Fe^{3+}	In active site of many redox enzymes and electron carriers; chlorophyll synthesis
Chlorine (Cl)	Cl^-	Photosynthesis; ion balance
Manganese (Mn)	Mn^{2+}	Activation of many enzymes
Boron (B)	B(OH)_3	Possibly carbohydrate transport (poorly understood)
Zinc (Zn)	Zn^{2+}	Enzyme activation; auxin synthesis
Copper (Cu)	Cu^{2+}	In active site of many redox enzymes and electron carriers
Nickel (Ni)	Ni^{2+}	Activation of one enzyme
Molybdenum (Mo)	MoO_4^{2-}	Nitrate reduction

Table 7.20 Essential nutrients supplied by liquid hydroponic fertilizers				
Element	Species required for plant uptake	Some commonly used ingredients	**Typical minimum concentration (ppm)	
Macronutrients			Grow	Bloom
Nitrogen (N)	Nitrate (NO_3^-)	Potassium nitrate, calcium nitrate, magnesium nitrate, ammonium nitrate	160	130
Phosphorus (P)	Phosphate (H_2PO_4^-)	Mono-potassium phosphate, mono-ammonium phosphate	30	60
Potassium (K)	Potassium (K^+)	Potassium nitrate, potassium sulfate	230	300
Calcium (Ca)	Calcium (Ca^{2+})	Calcium nitrate	80	80
Magnesium (Mg)	Magnesium (Mg^{2+})	Magnesium sulfate, magnesium nitrate	30	30
Sulfur (S)	Sulfate (SO_4^{2-})	Potassium sulfate, magnesium sulfate	60	60
Trace elements				
Iron (Fe)	Iron (Fe^{3+})	Iron EDTA, iron EDDHA	2.0	2.0
Copper (Cu)	Copper (Cu^{2+})	Copper EDTA	0.02	0.02
Manganese (Mn)	Manganese (Mn^{2+})	Manganese EDTA	0.5	0.5
Zinc (Zn)	Zinc (Zn^{2+})	Zinc EDTA	0.2	0.2
Molybdenum (Mo)	Molybdate (MoO_4^{2-})	Sodium molybdate	0.005	0.005
Boron (B)	Borate (H_2BO_3^-)	Borax	0.2	0.2

**Typical minimum concentration of elements available in high quality, general purpose hydroponic formulations during the growth and bloom phase (i.e. EC ~2.0mS)

Advantages of Hydroponic

- Plants can be grown anywhere as long as their growth requirements are met.
- It uses only 1/20th of water compared to traditional (soil based) gardening.
- It provides a sterile environment for plant production. This technique does not require pesticides, fertilizers and other chemicals, as there is no chance of damage due to soil-borne diseases or pests.
- Crops grow two times faster in hydroponic gardening. It provides controlled environment, and yield is doubled leading to more production from same amount of space.
- It needs 20% of less space in comparison to soil-based gardens, as plants with small roots can be grown closer to each other.
- Run-off in traditional gardening can lead to environment degradation due to high proportion of calcium, phosphorous and potassium content dissolved in it. But in hydroponic systems; water can be reused multiple times leading to water conservation with less expense incurred on it.
- There is no-doubt in the fact that hydroponics involves less labor. Upkeep is also minimal.
- It is simple to get complete control over nutrient balance by using solutions like Olivia's Growing Solution.
- There are no soil setup and testing hassles.

- Plants grown through this technique are healthy and have better nutritional value. It has been proved that vitamin content is 50% more in hydroponically grown plants as compared to conventional ones.
- It is easy to harvest in this type of gardening.
- There are no worries about the changing seasons, as crops can be grown all year round.
- Hydroponic gardening is amazingly stress-relieving and a relaxing hobby. Moreover, it is a great way to spend quality family time.

Disadvantages of Hydroponic Gardening

- Initial set up cost of hydroponic system is high. It requires constant supervision.
- These gardens can also become susceptible to power outage; in this case plants will dry out. If this ever happens, you have to manually water your garden.
- Water-based microorganism can be easily introduced.
- Technical knowledge is required for growing plants through hydroponics.

Few Plants Which Grown By Hydroponics

Lettuce, Tomatoes, Radishes, Celery, Cucumbers, Watermelon, Cantaloupe (Netted Melons) Strawberries, Blueberries, Grapes, Chives, Oregano, Basil, Sage, Rosemary

Plant Profile

Kingdom: Plantae
Unranked: Angiosperms
Unranked: Eudicots
Order: Caryophyllales
Family: Amaranthaceae
Subfamily: Chenopedioideae
Genus: *Spinacia*
Species: *S. Oleracea*



Botanical source: Spinach (*Spinacia oleracea*) is an edible flowering plant in the family Amaranthaceae native to central and western Asia. Its leaves are eaten as a vegetable.

Description

It is an annual plant (rarely biennial) growing to 30 cm (12 in) tall. Spinach may survive over winter in temperate regions. The leaves are alternate, simple, ovate to triangular, and very variable in size from about 2-30 cm (1-12 in) long and 1-15 cm (0.4-5.9 in) broad, with larger leaves at the base of the plant and small leaves higher on the flowering stem. The flowers are inconspicuous, yellow-green, 3-4 mm (0.1-0.2 in) in diameter, maturing into a small, hard, dry, lumpy fruit cluster 5-10 mm (0.2-0.4 in) across containing several seeds.

Distribution

Native to South- west Asia; cultivated throughout India

Cultivation: Harvest spinach when the leaves are tender and big enough to eat. Spinach is ready for picking 40 to 65 days after sowing. Cut spinach leaf by leaf-cut the outer leaves first allowing the inner leaves to grow larger—or cut away the whole plant one inch (2.5 cm) above the soil. Either way, the plant will keep producing new leaves as long as temperatures are cool. Spinach grows best between 60°

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and 65°F (15°-18°C)-commonly during spring or autumn. Plants commonly flower (bolt) and stop producing when temperatures reach the high 70°F (21°C). If temperatures rise into the 80°F (26°C), start picking outer leaves immediately; this will briefly delay bolting. Very warm temperatures turn spinach bitter. Overwintered spinach will give you an early spring harvest. Harvest spinach as close to meal time as possible for the best flavor. Cut spinach with a garden scissors or serrated bread knife. Store spinach cold and moist, 32°-40°F (0°-5°C) and 95 percent relative humidity. Place spinach in the refrigerator in a perforated plastic bag in the vegetable crisper section. Spinach will keep in the refrigerator for about 10 days. Spinach that is stored too cold or too long will develop brown spots on the midrib and the leaves will wilt and yellow.

Chemical constituents: Flavonoids: Spinaciaoleracea is very rich in the flavonoids. Various Flvonoids reported to be present Areque-retin; Myricetin; Kampeferol; Apigenin; Luteolin; Patuletin; Spinacetin; jaceidin; 4'-glu-curonide; 5,3',4'-trihydroxy-3-methoxy-6:7-methylenedioxyflavone-4'-glucuronide; 5,4'-dihydroxy-3,3'-dimethoxy-6:7-methylene dioxyflavone-4'-glu-curonide; 5,4'-dihydroxy-3,3'-dimethoxy-6,7-methylene-dioxi-flavone(C18H14O8.); 3,5,7,3',4'pentahydroxi-6-methoxiflavone.

Phenolic Compounds: The polyphenols isolated from the plant are para-coumaric acid, ferulic acid, ortho- coumaric acid. Carotinoids: Spinach shows presence of different carotinoids like lutein, β-carotene, violaxanthin and 9'-(Z)-neoxanthin Vitamins: Spinaciaoleracea contains high concentration of vitamin A, E, C, and K. and also folic acid, oxalic acid. Minerals: Along with these chemicals various minerals present in the spinach. These are magnesium, manganese, calcium, phosphorus, iron, zinc, copper and potash.

Traditional Uses

The plant is sweet, cooling, carminative, laxative, alexipharmac; useful in diseases of blood, brain, asthma, leprosy, biliousness; treatment of urinary calculi, hypoglycemic properties. emollient, wholesome, antipyretic, diuretic, maturant, laxative, digestible, anthelmintic, useful in urinary concretion, inflammation of the lungs and the bowels, sore throat, pain in joints, thirst, lumbago, cold and sneezing, sore eye, ring worm scabies, leucoderma, soalding urine, arrestvomiting , biliousness, flatulence. It have been used in the treatment of febrile conditions. The seeds are useful in fevers, leucorrhoea, urinary discharges, lumbago, and diseases of the brain and of the heart (Yunani). Seeds are laxative and cooling. They have been used in the treatment of difficulty in breathing, inflammation of the liver and jaundice. The green plant is given for the urinary calculi.

Instrumentation

Inductively Coupled Plasma\Optical Emission Spectroscopy

ICP/OES is one of the most powerful and popular analytical tools for the determination of trace elements in a myriad of sample types (Table 1). The technique is based upon the spontaneous emission of photons from atoms and ions that have been excited in a RF discharge. Liquid and gas samples may be injected directly into the instrument, while solid samples require extraction or acid digestion so that the analytes will be present in a solution.

The sample solution is converted to an aerosol and directed into the central channel of the plasma. At its core, the inductively coupled plasma (ICP) sustains a temperature of approximately 10000K, so the aerosol is quickly vaporized. Analyte elements are liberated as free atoms in the gaseous state. Further collisional excitation within the plasma imparts additional energy to the atoms, promoting them to excited states. Sufficient energy is often available to convert the atoms to ions and subsequently promote the ions to excited states. Both the atomic and ionic excited state species may then relax to the ground state via the emission of a photon. These photons have characteristic energies that are determined by the quantized energy level structure for the atoms or ions. Thus, the wavelength of the photons can be used to identify the elements from which they originated. The total number of photons is directly proportional to the concentration of the originating element in the sample. The instrumentation associated with an ICP/OES system is relatively simple. A portion of the photons emitted by the ICP is collected with a lens or a concave mirror. This focusing optic forms an image of the ICP on the entrance

aperture of a wavelength selection device such as a monochromator. The particular wavelength exiting the monochromator is converted to an electrical signal by a photo detector. The signal is amplified and processed by the detector electronics, then displayed and stored by a personal computer.

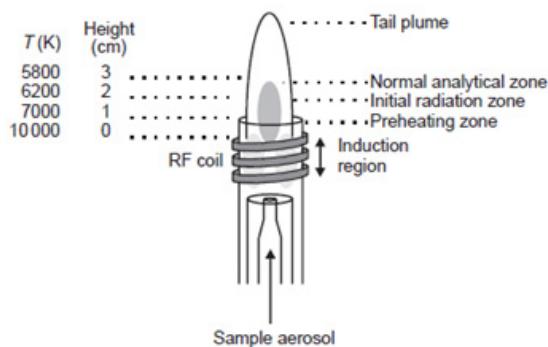
Table 1 Survey of elemental application areas of ICP/OES⁽¹⁻³⁾

Categories	Examples of samples
Agricultural and food	Animal tissues, beverages, feeds, fertilizers, garlic, nutrients, pesticides, plant materials, rice flour, soils, vegetables, wheat flour
Biological and clinical	Brain tissue, blood, bone, bovine liver, feces, fishes, milk powder, orchard leaves, pharmaceuticals, pollen, serum, urine
Geological	Coal, minerals, fossils, fossil fuel, ore, rocks, sediments, soils, water
Environmental and water	Brines, coal fly ash, drinking water, dust, mineral water, municipal wastewater, plating bath, sewage sludge, slags, seawater, soil
Metals	Alloys, aluminum, high-purity metals, iron, precious metals, solders, steel, tin
Organic	Adhesives, amino acids, antifreeze, combustion materials, cosmetics, cotton cellulose, dried wood, dyes, elastomers, epoxy, lubricant, organometallic, organophosphates, oils, organic solvent, polymers, sugars
Other materials	Acids, carbon, catalytic materials, electronics, fiber, film, packaging materials, paints and coatings, phosphates, semiconductors, superconducting materials

The characteristics of the ICP as an analytical atomic emission source are so impressive that virtually all other emission sources [such as the flame, microwave induced plasma (MIP), direct current plasma (DCP), laser-induced plasma (LIP), and electrical discharge] have been relegated to specific, narrowly defined application niches. Indeed, even much of the application field originally assigned to atomic absorption spectrometry (AAS), using both the flame and graphite furnace atomic absorption spectrometry (GFAAS), has been relinquished to the ICP. Compared to these other techniques, ICP/OES enjoys a higher atomization temperature, a more inert environment, and the natural ability to provide simultaneous determinations for up to 70 elements. This makes the ICP less susceptible to matrix interferences, and better able to correct for them when they occur. In cases where sample volume is not limited, ICP/OES provides detection limits as low as, or lower than its best competitor, GFAAS, for all but a few elements. Even for these elements, the simplicity with which the ICP/OES instrument is operated often outweighs the loss in sensitivity.

Inductively coupled plasma operation

As shown in Figure the so-called ICP “torch” is usually an assembly of three concentric fused-silica tubes. These are frequently referred to as the outer, intermediate, and inner gas tubes. The diameter of the outer tube ranges from 9 to 27 mm. A water-cooled, two- or three-turn copper coil, called the load coil, surrounds the top section of the torch, and is connected to a RF generator. The outer argon flow (10–15 L min⁻¹) sustains the high temperature plasma, and positions the plasma relative to the outer walls and the induction coil, preventing the walls from melting and facilitating the observation of emission signals. The plasma under these conditions has an annular shape. The sample aerosol carried by the inner argon flow (0.5–1.5 L min⁻¹) enters the central channel of the plasma and helps to sustain the shape.



The intermediate argon flow (0-1.5 Lmin⁻¹) is optional and has the function of lifting the plasma slightly and diluting the inner gas flow in the presence of organic solvents. The ICP is generated as follows. RF power, typically 700-1500W, is applied to the load coil and an alternating current oscillates inside the coil at a rate corresponding to the frequency of the RF generator. For most ICP/OES instruments, the RF generator has a frequency of either 27 or 40 MHz. The oscillation of the current at this high frequency causes the same high-frequency oscillation of electric and magnetic fields to be set up inside the top of the torch. With argon gas flowing through the torch, a spark from a Tesla coil is used to produce "seed" electrons and ions in the argon gas inside the load coil region. These ions and electrons are then accelerated by the magnetic field, and collide with other argon atoms, causing further ionization in a chain reaction manner. This process continues until a very intense, brilliant white, teardrop shaped, high-temperature plasma is formed. Adding energy to the plasma via RF-induced collision is known as inductive coupling, and thus the plasma is called an ICP. The ICP is sustained within the torch as long as sufficient RF energy is applied. In a cruder sense, the coupling of RF power to the plasma can be visualized as positively charged Ar ions in the plasma gas attempting to follow the negatively charged electrons flowing in the load coil as the flow changes direction 27 million times per second.

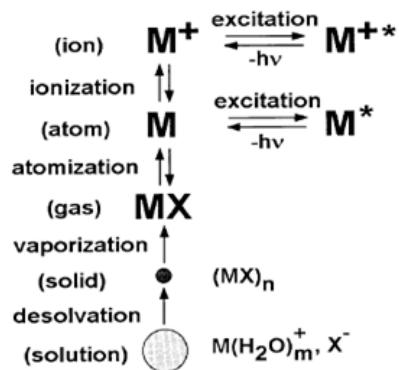
The temperature gradient within the ICP with respect to height above the load coil. It also gives the nomenclature for the different zones of the plasma as suggested by Koirtyohann., *et al.* 7/The induction region (IR) at the base of the plasma is "doughnut-shaped" as described above, and it is the region where the inductive energy transfer occurs. This is also the region of highest temperature and it is characterized by a bright continuum emission. From the IR upward towards to the tail plume, the temperature decreases. An aerosol, or very fine mist of liquid droplets, is generated from a liquid sample by the use of a nebulizer. The aerosol is carried into the center of the plasma by the argon gas flow through the IR. Upon entering the plasma, the droplets undergo three processes. The first step is desolvation, or the removal of the solvent from the droplets, resulting in microscopic solid particulates, or a dry aerosol. The second step is vaporization, or the decomposition of the particles into gaseous state molecules. The third step is atomization, or the breaking of the gaseous molecules into atoms. These steps occur predominantly in the preheating zone (PHZ). Finally, excitation and ionization of the atoms occur, followed by the emission of radiation from these excited species. These excitation and ionization processes occur predominantly in the initial radiation zone (IRZ), and the normal analytical zone (NAZ) from which analytical emission is usually collected. 1/.

Inductively Coupled Plasma Characteristics

The main analytical advantages of the ICP over other excitation sources originate from its capability for efficient and reproducible vaporization, atomization, excitation, and ionization for a wide range of elements in various sample matrices. This is mainly due to the high temperature, 6000-7000K, in the observation zones of the ICP. This temperature is much higher than the maximum temperature of flames or furnaces (3300K). The high temperature of the ICP also makes it capable of exciting refractory elements, and renders it less prone to matrix interferences. Other electrical-discharge-based sources, such as alternating current and direct current arcs and sparks, and the MIP, also have high temperatures for excitation and ionization, but the ICP is typically less noisy and better able to handle liquid samples. In addition, the ICP is an electrode less source, so there is no contamination from the impurities present in an electrode material. Furthermore, it is relatively easy to build an ICP assembly and it is inexpensive, compared to some other sources, such as a LIP. The following is a list of some of the most beneficial characteristics of the ICP source.

High temperature (7000–8000k), high electron density (10¹⁴-10¹⁶ cm⁻³), appreciable degree of ionization for many elements , simultaneous multi element capability (over 70 elements including p and s) ,Low background emission, and relatively low chemical interference ,high stability leading to excellent accuracy and precision , .excellent detection limits for most elements (0.1-100 ngml) . Wide linear dynamic range (ldr) (four to six orders of magnitude), applicable to the refractory elements. Cost-effective analyses.

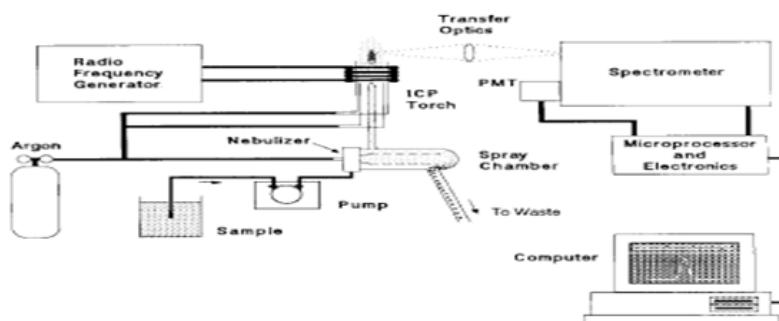
Principle



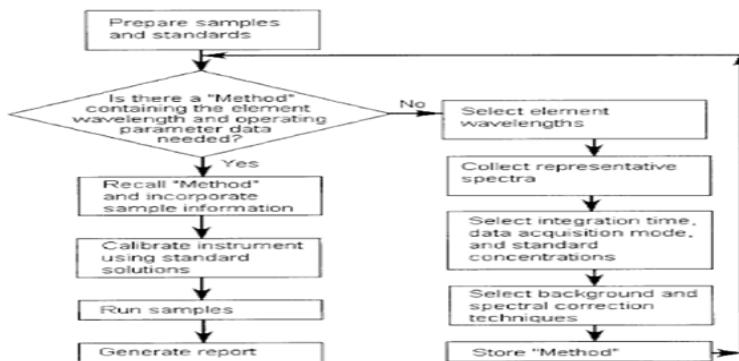
Instrumentation



Process



Process



Applications

It is mainly used to estimate metals in following felids;

- Agricultural& food
- Biological& clinical
- Geological
- Environmental & water
- Pharma drugs

Aim and objective

Agriculture is the key development in the rise of sedentary human civilization, whereby farming of domesticated species created food surpluses that nurtured the development of civilization. In Modern agronomy, plant breeding, agrochemicals such as pesticides and fertilizers, and technological developments have in many cases sharply increased yields from cultivation, but at the same time have caused widespread ecological damage and negative human health effects. Despite what government agencies and corporations tell you, pesticide products currently on the market are not safe, even when they are used legally. There are many flaws in the way that pesticides are registered and in our political process that allows corporations to influence pesticide policy to allow the continued use of their poisonous products. Since the publication of Rachel Carson's landmark 1962 book Silent Spring, the impacts of pesticides on the environment have been well known. Pesticides are toxic to living organisms. Some can accumulate in water systems, pollute the air, and in some cases have other dramatic environmental effects. Scientists are discovering new threats to the environment that are equally disturbing.

Pesticide use can damage agricultural land by harming beneficial insect species, soil microorganisms, and worms which naturally limit pest populations and maintain soil health; Even if we know that a pesticide causes severe health and environmental impacts, including cancer and genetic damage, it may still be allowed for use. The EPA may determine that a cancer-causing chemical may be used despite its public health hazard if its "economic, social or environmental" benefits are deemed greater than its risk. According to the US EPA, more than 70 active ingredients known to cause cancer in animal tests are allowed for use.

Agricultural food production and water management are increasingly becoming global issues that are fostering debate on a number of fronts. Significant degradation of climatic conditions, land and water resources, including the depletion of aquifers, has been observed in recent decades, and the effects of global warming on agriculture. In view of health hazards posed by indiscriminate use of chemical fertilizers and pesticides, extremely difficult situations of land and water managements and adverse influence of climatic conditions such as heavy rains, air pollution, water pollution, soil pollution, heavy sun effect) on the quality and quantity of agricultural products ,we have panned the cultivation of spinach hydroponically.

The objective of present study was to compare the phytochemical constituents of geoponic and hydroponic cultivated spinach and screening for antioxidant activity based on their phenolic and flavonoid contents .Further, how much extent the hydroponic cultivation is influencing the presence of metals by carrying out metal analysis by Inductively Coupled Plasma/Optical Emission Spectrometry.

Literature Review

About spinach

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Plan of work

1. Designing and fabricating a mini NFT system
2. Collection and purchase of spinach seeds.
3. Procurement of hydroponics nutrition medium, mineral water, pots, LED, Metal halide light and chemicals.
4. Germination
5. Plantation/cultivation of spinach in NFT system
6. Study pH, TDS, conductivity, light intensity, humidity and relative humidity for 2 times a day.
7. Measurement of the growth of spinach.
8. Extraction with Ethanol for both hydroponics and geoponics
9. Phytochemical screening
10. Estimation of total phenolic and total flavonoid content in the extract
11. Estimation of metals by ICPOES

Method and Methodology

Materials Required

Chemicals Required: Ethanol, Milli Q water, Standard Reagent Bottles 1000ppm, Conc Nitric Acid.

Instruments Required ICPOES, UV spectroscopy, pH & conductivity meter, TDS meter, luxmeter, hygrometer, Heating Mantles, Hot plate

Glassware Required: Beaker (50 ml & 100 ml), Volumetric Flasks (20 ml, 25 ml, 50 ml & 100 ml), Glass Rods, Micro Pipettes, Pipettes (1 ml, 5 ml, 10 ml), (500 ml) Round Bottom Flask and Condenser.

Citation: Chandaka Madhu M., et al. "Comparitive Studies on Phytochemical Screening, Total Phenolic, Total Flavonoid Content and Metal Analysis of Hydroponic (Nft System) and Geoponic Cultivated Spinach". *Chronicles of Pharmaceutical Science* 1.2 (2017): 89-113.

Micillaneous: Test tube stand, test tube holders, filter paper, butter paper, spatula, thermometers, stands, tissue paper, zip pouches, markers, gloves, labels, cotton swabs, disinfectant etc.

Instrumentation of nft



Cultivation of spinach by hydroponics technique

Germination: the seeds of spinach, vermicompost, cocoa pit and colonies were purchased from local store. The colonies were filled with vermicompost and cocoa pit with the ratio of 30:70. The seed were sown at a depth of 1 cm and it was covered. The water was sprinkled twice a day and the colonies were placed in dark place for a week.

Plantation and conditions for nft system

Firstly an young plant of spinach is taken from colonies. The cocoa pit and vermicompost are thoroughly washed in a beaker. After washing the roots clearly, the plant is placed in to a basket and mud rocks are introduced into the basket which helps the plant to stand still in basket. This basket is introduced into the NFT system.

Preparation of powder

After 30 days weeks. The cultivated plant material of *spinach* (hydroponics) and marketed spinach (geponics) *which was* procured from local market were shade dried and then powdered with a mechanical grinder to obtain a coarse powder. The powder was passed through sieve no 40 and was stored in an air tight container until further use. The powder was used for the extraction process.

Preparation of alcholic extract

The ethanoic extract of the plant was prepared using hot condensation process. The coarse powders of both hydroponic plant and geoponic plant 200g each were taken in separate round bottom flasks and ethanol was poured until the powder was soaked. Then refluxed it for 8 hrs at 25c. After 8 hrs, the suspension was filtered through a fine muslin cloth.

The solvent was removed by heating until plant extracts were obtained and then the percentage yields were calculated.

Plant	Light Conditions	Hid lamp type	Favorable temp	Ph	Ppm	Tds
Spinach	medium	400/1000W	Cool to warm	6.0-7.0	1260	1610

Phytochemical Screening

S. No	Phytochemical	Name of the Test
1.	Glycosides	Legals test
2	Saponins	Froth test
3	Alkaloids	Dragendorffs test
4	Tannins	Chromic test
5	Flavoinids	Shinoda test
6	Mucilage	Ruthenium red test
7	Carbohydrates	Molisch test
8	Proteins	Xanthoproteic test
9	Phytosterols	Salkowski test

Determination of Total Phenolic Content: [46]

Total Phenolic content of the extract was determined by Folinciocalteau reagent according to Singleton and Rossi using Gallic acid as a standard. The concentration of total phenols was expressed in terms of mg of Gallic Acid equivalents per gram of extract.

Plotting of calibration curve

The stock solution of gallic acid (1000 µg/ml) was diluted to get the various concentrations 10,20,30,40,50,60,70 and 80 µg/ml by diluting with distilled water. About 0.5 ml of Folinciocalteau reagent was added to each test tube, mixed thoroughly and incubated for 3 min at room temperature. Later 3 ml of 20% Na₂CO₃ was added and mixed thoroughly and incubated in boiling water bath for 1 min. The absorbance was measured at 650 nm and the observations are shown in table: 3. The standard plot was drawn (absorbance Vs concentration.) The amount of total phenolic content s was estimated in both Geponics and Hydroponics extracts of spinach.

Determination of Total Flavanoid Content: [47]

Total Flavanoid content was measured by the aluminium chloride colorimetric assay. Standard solution of catechin (20, 40, 60, 80 and 100 µg/ml) was taken in separate 10 ml volumetric flasks containing 4 ml of distilled water. 0.3 ml of 5% NaNO₂ was added. After 5 min, 0.3 ml 10% AlCl₃ was added. At 6th min, 2 ml of 1M NaOH was added and the total volume was made up to 10 ml with distilled H₂O. The solution was mixed well and the absorbance was measured against prepared reagent as blank at 510 nm and recorded in a suitable table. Table No: 4. A standard plot was drawn (absorbance Vs concentration. Figure No:

An aliquot (1 ml) of extracts were taken and colour developed in the same procedure and absorbance were measured and the standard plot was drawn (Absorbance Vs concentration.) The amounts of flavonoids are calculated and expressed as mg catechin equivalents (CE)/g of extract from the interpolation of the curve. Samples were analyzed in duplicates.

Procedure for estimation of metals

Preparation of extract sample (acid digestion)

1g of the extract was weighed and transferred into 50 ml of beaker. Then add 5 ml of conc. HNO_3 and placed it on hot plate until the organic fumes were completely stopped. Then 25 ml of water was added for acid digestion on hot plate. Digestion to be taken until 50% of the sample was too evaporated and remaining sample was filtered and made up to 25 ml, and subjected to furthered dilutions.

Preparation of standard

The standard reagent 1000 ppm was purchased from The National Institute of Standards and Technology (NIST) from Pune. From the standard reagent (1000 ppm) furthered dilutions were made containing 1,2,3 ppm. Blank, Standards (0.5,1,1.5 ppm), Blank and Sample were aspirated into ICPOES.

Caluculation

$$\text{The amount metals(ppm)} = \frac{\text{conc of the test} \times \text{dilution factor}}{\text{weight of the sample}}$$

Results and Discussion

1. Design of nft system



2. Germination For 1 Week in Dark



B. After 2 weeks



3. Introducing plant into nft:



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Study ph, tds, Conductivity, Light intensity, Humidity and Relative Humidity for 2 times a day:

	Morng	Morng	Morng	Morng	Morng	Evng	Evng	Evng	Evng	Evng
Date	Temp	humidity	Lux	Ph	Cond	Temp	Humidity	Lux	Ph	Cond
27/01/17	25.9	48%	10000	5.7	1610	23.2	28%	6000	5.8	1610
28/01/17	25	47%	10004	5.9	1611	23.4	27%	6045	5.7	1608
29/01/17	26.7	49%	10023	5.7	1608	24.6	27%	6000	5.8	1608
30/01/17	28.7	47%	10014	5.9	1610	26.4	28%	6004	5.6	1602
31/01/17	25	46%	10004	6.0	1611	23	28%	6004	6.2	1610
01/02/17	26.7		10004	6.2	1611	24	28%	6045	6.3	1605
02/02/17	28	48%	10016	6.0	1612	25	27%	6003	5.8	1604
03/02/17	25	46%	10023	5.7	1611	23	28%	6000	6.2	1608
04/02/17	25.9	48%	10004	5.9	1610	26	28%	6045	6	1610
05/02/17	25	46%	10004	6.2	1608	24.5	27%	6004	5.7	1608
06/02/17	26.7	47%	10014	5.7	1611	23.2	28%	6000	5.8	1604
07/02/17	25	46%	10014	5.9	1608	26.5	28%	6045	6.2	1608
08/02/17	26	46%	10023	6.2	1610	24.2	27%	6009	6.2	1610
09/02/17	25.9	48%	10023	6.2	1611	23.5	28%	6004	6	1605
10/02/17	25	49%	10023	5.7	1608	22.4	27%	6000	5.7	1604
11/02/17	26.7	49%	10014	5.9	1610	22.3	28%	6045	5.8	1610
12/02/17	25.9	48%	10023	6.2	1608	26.5	28%	6009	6.2	1608
13/02/17	25	49%	10014	6.2	1611	24.3	27%	6004	5.7	1604
14/02/17	27	47%	10023	5.7	1610	23.6	28%	6000	5.8	1605
15/02/17	25	47%	10023	5.9	1608	22.6	28%	6045	6.2	1605
16/02/17	26.7	48%	10014	6.2	1610	22.3	27%	6004	5.8	1604
17/02/17	25.9	48%	10000	5.7	1611	22.7	28%	6000	6	1610
18/02/17	27	49%	10014	5.9	1608	26.5	28%	6045	5.7	1608
20/02/17	25.9	48%	10000	6.2	1608	24.6	27%	6009	5.8	1604
21/02/17	25	47%	10014	6.2	1610	26.7	28%	6045	6	
22/02/17	25.9	48%	10014	5.7	1611	24.7	28%	6004	5.8	1608
24/02/17	25	47%	10004	5.9	1608	23.5	27%	6000	6	1604
25/02/17	26.7	49%	10000	5.8	1610	22.6	27%	6045	5.7	1610
26/02/17	25	47%	10004	5.7	1608	26.4	27%	6009	5.8	1605

Measurement of the growth of spinach:

Date: 27/01/17

S. No	Plant	Activity	Condition	Height (cm)	Pigmentation
01	01	No activity	Dryness	5	-
02	02	Root elongation	Normal	5.5	-
03	03	Rich root elongation	Normal	7.5	Light yellow
04	04	Root elongation	Normal	6	-

Citation: Chandaka Madhu M., et al. "Comparitive Studies on Phytochemical Screening, Total Phenolic, Total Flavoid Content and Metal Analysis of Hydroponic (Nft System) and Geoponic Cultivated Spinach". *Chronicles of Pharmaceutical Science* 1.2 (2017): 89-113.

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05	05	Root elongation	Normal	5.5	Light yellow
06	06	Root elongation	Normal	7	-
07	07	Root elongation	Normal	6	-
08	08	-	Normal	4	-
09	09	Weak	Dryness	4.5	-
10	10	Weak	Living	5	Colour change
11	11	Rich root elongation	Normal	13	-
12	12	Slight root elongation	Normal	6.5	-
13	13	-	Normal	6	-
14	14	Weak	Dryness	-	-
15	15	Root elongation	Normal	6.3	-
16	16	-	Normal	7	-
17	17	-	Normal	6	-
18	18	-	Normal	5.8	Slight yellow
19	19	Weak	Dryness	-	-
20	20	Weak	Normal	5.4	-

Date: 02/02/17

S. No	Plant	Activity	Condition	Height (cm)	Pigmentation
01	01	Dead	-	-	-
02	02	Elongation of roots	normal	7	-
03	03	Elongation of roots	normal	6.2	-
04	04	Elongation of roots	normal	7	-
05	05	Elongation of roots	normal	6.5	-
06	06	Elongation of roots	normal	7.5	-
07	07	Elongation of roots	normal	-	-
08	08	-	Weak	-	Yellow
09	09	Dead	-	-	-
10	10	Weak	weak	-	Yellow
11	11	Elongation of roots	normal	13.6	Dark green
12	12	-	normal	6	-
13	13	Dead	-	-	-
14	14	Weak	-	-	Light yellow
15	15	Elongation of leaves	active	6.4	-
16	16	Active	Active	7	-
17	17	-	Normal	-	Slight yellow
18	18	Dead	-	-	-
19	19	Dead	-	-	-
20	20	Elongation of roots	Normal	6	-

Citation: Chandaka Madhu M., et al. "Comparative Studies on Phytochemical Screening, Total Phenolic, Total Flavoid Content and Metal Analysis of Hydroponic (Nft System) and Geoponic Cultivated Spinach". *Chronicles of Pharmaceutical Science* 1.2 (2017): 89-113.

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Date: 08/02/17

S. No	Plant	Activity	Condition	Height (cm)	Pigmentation
01	01	Roots initiation	Normal	-	-
02	02	Elongation of roots	Normal	8	Dark Brown
03	03	Elongation of roots	Normal	7	Light Brown
04	04	Elongation of roots	Normal	8.6	Dark Brown
05	05	Elongation of roots	Normal	7.8	Light Brown
06	06	Elongation of roots	Normal	5	Light Brown
07	07	Roots Intiation	Normal	-	-
08	08	Elongation of roots	Normal	5	Light Brown
09	09	Elongation of roots	Normal	6	Light Brown
10	10	Weak	Weak	-	Light Yellow
11	11	Elongation of roots	Normal	14	Dark Brown
12	12	Elongation of roots	Normal	5	Light Brown
13	13	Elongation of roots	Normal	7	Light Brown
14	14	Roots Initiation	Normal	-	-
15	15	Elongation of roots	Normal	5	Light Green
16	16	Dead	-	-	-
17	17	Elongation of roots	Normal	7	Dark Brown
18	18	Roots initiation	Normal	-	-
19	19	Roots initiation	Normal	-	-
20	20	Elongation of Roots	Normal	6	Light Brown

Date: 14/02/17

S. No	Plant	Activity	Condition	Height (cm)	Pigmentation
01	01	Dead	-		-
02	02	Normal	Normal	7.2	Normal
03	03	Root elongation	Normal	9.2	Normal
04	04	Root elongation	Normal	7	-
05	05	Weak	Weak	3	Yellow
06	06	Root elongation	Rich	12	Normal
07	07	Root elongation	Normal	6	Normal
08	08	Survived	Normal	4	Normal
09	09	Root elongation	Normal	7	Normal
10	10	Moderate death	Weak	3	Yellowish pink
11	11	Root elongation	Normal	12	Normal
12	12	Root elongation	Normal	5.5	Normal
13	13	Normal	Week	6	Normal
14	14	Dead	-	-	-
15	15	Maximum death	-	-	-

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16	16	Normal	Normal	7.2	Normal
17	17	Survived	Normal	4.9	Normal
18	18	Normal	Normal	5	Normal
19	19	Dead	-	-	-
20	20	Normal	Normal	5	Normal

Date: 14/02/17

S. No	Plant	Activity	Condition	Height (cm)	Pigmentation
01	01	Weak	Weak	4	Yellow colour
02	02	Normal	Normal	5.5	Yellow spots
03	03	Root elongation	Active	5.7	-
04	04	Root elongation	Normal	6	-
05	05	Normal	Normal	6.5	Yellow colour
06	06	Normal	Normal	5.5	-
07	07	Elongation of roots and leaves	Active	8.7	Dark green
08	08	Weak	About to death	4	Yellow colour
09	09	Normal	Normal	6.5	-
10	10	Normal	Normal	6	-
11	11	Weak	About to death	4.5	Yellow colour
12	12	Active	Active	9	-
13	13	Root elongation	Active	8.8	-
14	14	Rich root elongation	Active	14	Dark green
15	15	Elongation of roots	Active	14	-
16	16	Elongation of roots and leaves	Active	16	-
17	17	Weak	About to death	4	Dryness
18	18	Normal	Normal	7	Yellow
19	19	Normal	Normal	7.2	-
20	20	Elongation of roots	Active	9.7	Dark Brown

Date: 26/02/17

S. No	Plant	Activity	Condition	Height (cm)	Pigmentation
01	01	Weak	Normal	4.5	-
02	02	Normal	Normal	5.5	Yellow spots
03	03	Root elongation	Active	5.7	-
04	04	Root elongation	Normal	6.1	-
05	05	Normal	Normal	6.5	-
06	06	Normal	Normal	5.5	-
07	07	Elongation of roots and leaves	Active	9	Dark green

Citation: Chandaka Madhu M., et al. "Comparative Studies on Phytochemical Screening, Total Phenolic, Total Flavoid Content and Metal Analysis of Hydroponic (Nft System) and Geoponic Cultivated Spinach". *Chronicles of Pharmaceutical Science* 1.2 (2017): 89-113.

08	08	Weak	Weak	4.3	Yellow colour
09	09	Normal	Normal	6.6	-
10	10	Normal	Normal	6.2	-
11	11	Weak	Weak	4.5	Yellow colour
12	12	Active	Active	9.4	-
13	13	Root elongation	Active	8.5	-
14	14	Rich root elongation	Active	15	Dark green
15	15	Elongation of roots	Active	14	-
16	16	Elongation of roots and leaves	Active	16	-
17	17	Weak	About to death	4.1	Dryness
18	18	Normal	Normal	7.1	Yellow
19	19	Normal	Normal	7.4	-
20	20	Normal	Active	9.7	Dark Brown

Percentage yield of the extract

S. No	Name of The Plant	Percentage Yield (%)
1	Hydropoinc extract	12.6%
2	Geopoincs extract	17.4%

Table 1:

Preliminary Phytochemical Screening

Tests for	Hydropoincs Extract	Geoponic Extract
Carbohydrates	+	+
Glycoside	+	+
Saponins	+	+
Alkaloids	-	-
Phytosterols	-	-
Fixed Oils	+	+
Gums and Mucilage	-	-
Proteins	-	-
Phenolic compounds and Tannins	+	+
Flavonoids	+	+

Table 2: Data showing the preliminary phytochemical screening of extract of spinach (+ = present - = absent).

Estimation of Total Phenolic Content

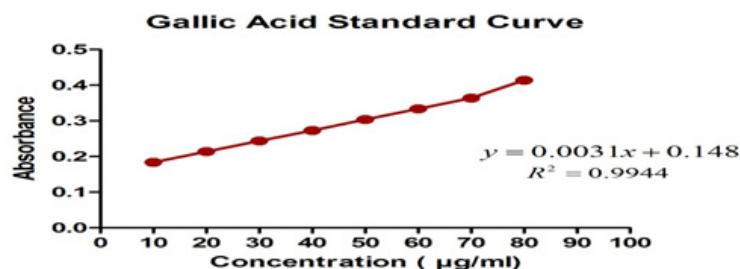
Standard (Gallic acid) Calibration Curve	
Concentration ($\mu\text{g/ml}$)	Absorbance
10	0.184
20	0.214
30	0.244
40	0.273
50	0.304
60	0.334
70	0.364
80	0.414

Table 3: Data showing absorbance of various concentration of Gallic acid.

Sample	
Concentration (100 $\mu\text{g/ml}$)	Absorbance
Geoponics extract	0.166
Hydroponics extract	0.184

Total Phenolic Content

Graph -1



From the Standard Graph of Gallic Acid, The total phenol concentration present in the Geoponics and Hydroponics extract was found to be:

Geoponics Extract: 58 mg GAE/g of extract

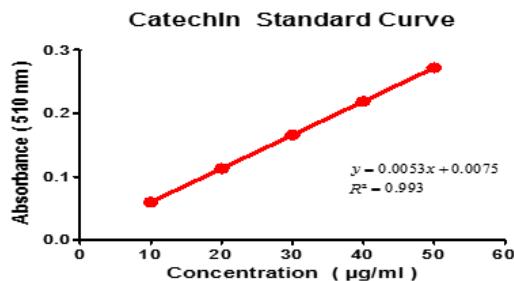
Hydroponics Extract: 116.1 mg GAE/g of extract

Total Flavanoid Content

Catechin Standard Curve	
Concentration ($\mu\text{g/ml}$)	Absorbance
10	0.060
20	0.113
30	0.166
40	0.219
50	0.272

Table 4: Data showing absorbance of various concentration of Catechin.

Sample Solution	
Hydroponics Extract (100 $\mu\text{g/ml}$)	0.138
Geoponics Extract (100 $\mu\text{g/ml}$)	0.115



From the Standard Graph of Catechin, The total flavanoid concentration present in the geoponics and Hydroponics extract was found to be: Geoponics Extract: 202.8 mg of CE/g of extract
Hydroponics Extract: 242.6 mg of CE/g of extract

Metal Analysis by Using Icpoes for Hydropoines and Geopoines for 1 Gm of Extract

Name of the Metal	Hydroponics Extract (Conc of Metals in ppm)	Geoponics Extract (Conc of Metals in ppm)
Arsenic	Not detected	1.12
Calcium	370.70	649.73
Cadmium	4.07	0.42
Chromium	3.06	3.48
Cobalt	0.39	0.40
Copper	6.57	2.27
Iron	16.61	31.73
Manganese	8.92	4.96
Nickel	2.41	1.54
Lead	Not detected	0.54

Vanadium	Not detected	Not detected
Zinc	13.83	6.20
Sodium	800	400
Potassium	5300	3800

Discussion

Our experiment to cultivate spinach by hydroponics has provided some significant observations. The percentage yield of ethanol extract in hydroponics is relatively low (Hydroponic extract is 12.6% whereas *Geoponics* extract was 17.4%). However, there was no variation in the nature of phytoconstituents present in them.

But quantitative estimation of total phenolics by Folinciocalteau method proved that the Hydropoinc extract have more amount of total phenolics in comparison to conventional method of cultivated sample. (*Geopoins* Extract: 58 mg GAE/g of extract and *Hydropoins* Extract: 116.1 mg GAE/g of extract)

Similarly, the total flavanoid concentration present in the geponics and Hydroponics extract s were compared. They were found to be 202.8 mg of CE/g of extract geponics Extract and 242.6 mg of CE/g of extract of Hydropoins. Therefore it is assumed that polyphenolics are excellent antioxidants and free radical scavengers, the spinach cultivated by hydroponics has better medicinal value. Metal analysis by by using ICPOES has proved that the spinach cultivated by hydropoins method is totally free from toxic arsenic and lead. Copper, Cadmium, sodium, potassium and manganese levels are relatively high.

In view of the promising results obtained, further research may be envisaged to understand the role of nutrient medium in enhancing the beneficial contents like iron

Conclusion

The final conclusion is we successfully achieved to produce a healthy organic plant of spinach having 0% of heavy metal content, 0% usage of pesticides or insecticides and other fertilizers.

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