

Influence of Spent Mushroom Waste on the growth of *Spinacia oleracea* L.

BY

ABITHAKUJALAMBALS

19PBO002

A Dissertation submitted to the
Avinashilingam Institute for home Science and Higher Education for Women
Coimbatore-641-043

In Partial Fulfillment of the Requirements for the degree of
DEGREE OF MASTER OF SCIENCE IN BOTANY

MAY 2021

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**Signature of the
Head of the Department**



Signature of supervisor

Acknowledgement

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Introduction

INTRODUCTION

Nowadays, there are many agricultural wastes reused in various ways. One such useful option is using them for composting or using it as an organic manure. Eventually, many waste substrates of different kinds are used in Gardening and also few agricultural wastes are used to grow Mushrooms. The agricultural wastes used as substrates can be reused after composting as organic manure. Compost is created by combining organic wastes such as wasted food, yard trimmings and manures, in the right ratio into piles, rows or vessels/allowing the finished material to fully stabilize and mature through a curing process. The usage of such compost to soil improves the physical properties of the soil.

Organic waste in landfills produces methane, by composting wasted foods and organics, methane emission is significantly reduced, it also reduces the need of chemical fertilizers, it also promotes the yield of agricultural crops. It also leads to aid reforestation, wetland restoration, habitat revitalization, and efforts by improving contaminated, compacted, and marginal soils. It also helps in water retention in soils. Based on this there are different forms of compost vermicomposting, organic waste etc. On the basis of this Spent Mushroom Compost (SMC) has been formed, it is formed from agricultural waste used for cultivation of mushrooms by mushroom industry and are produced abundantly.

SMC produced can be discarded or burned. But discarding and burning generates only 10% of energy and also brings about pollution. In China the SMC is not considered as waste, and they have developed several methods to utilize these SMC. Whereas in Malaysia most of the mushroom waste is collected and especially from grey oyster mushroom because it is a good source of proteins, vitamins and, minerals, it is good source of food and medicine.

Because of the less toxic or non-toxicity nature of the substrates, these materials could be employed as soil amendment for different crops. The spent substrate from mushrooms could be analysed and found it could be nutrient rich with composition of NPK contents, and it also has high cation exchange capacity. Therefore, it may also have the capability to replace the inorganic farm yard manure for the cause of growing horticultural and cereal crops. It also plays a role of feeding material for vermicomposting. Generally, there may be two types of spent substrate:

1. Fresh SMC – SMC applied to plants immediately after removing from mushroom farms

2. Weathered SMC – SMC undergo few weeks of decomposition before utilization by farmers.

Application of SMC could increase the activity of soil microorganisms and earthworms, which in turn helps to improve soil porosity and water retaining capacity. Working with SMC is more effective and beneficial compared to other fertilizers in case of poor soil. Other than many elements SMC contains many hypha proteins because of the decomposition of cellulose and lignin components which prevents the deficiency of Nitrogen. Unlike the other manures it could be Eco friendly to fulfil the utilization of natural resources. These composts are also used as substrate for potting plants without soil. Other than SMC there are furthermore, species are there which act as a plant growth promoting microorganism such as *Azospirillum spp*, *Phosphobacteria* etc.

More than 20 species of *Azospirillum* are used in Agriculture. *Azospirillum* are gram-negative free-living nitrogen fixing bacteria. They are generally isolated from the rhizosphere of many grasses and many cereals all around the world including tropical and temperate regions. *Azospirillum* could positively influence plant growth, crop yield, and nitrogen content. It includes some other biological functions such as nitrogen fixation and auxin production.

Phosphobacteria have the ability to convert insoluble compounds of phosphorus into available phosphate that enhance the availability to plants (Rodriguez and Froga,1999, Barea *et al.*, 2005, Son *et al.*, 2006, Souchie *et al.*, 2006 and Lugo *et al.*, 2008). These microorganisms and mushroom spent increases the growth and yield in plants. Leafy vegetables are used to study the improvement in growth and other parameters as both SMC and the microorganisms have beneficial effects in growing plants. The objective of the present study is

1. To compare the growth using fresh SMC and *Azospirillum* and *Phosphobacteria* by growing leafy vegetable (*Spinacia olearacea*).
2. To analyse the phytochemical content - chlorophyll present in leafs of plants grown under various treatments.
3. To analyse the elements present in the soil in 0th and 30th day of various treatments.

Review of Literature

REVIEW OF LITERATURE

Agriculture in earlier days were based on organic farming. In order to increase the yield, chemical fertilizers were used. Nowadays, farmers are using organic manure to grow crops. Organic manures are prepared by composting various agricultural wastes. Spent Mushroom waste is a kind of compost which is obtained from the remaining obtained after harvesting mushrooms.

Spent Mushroom Waste

Worldwide, mushrooms are cultivated using agricultural wastes and woods (Danny, 2002). Some of the agricultural wastes include paddy straw, wheat straw, corn leaves and husks, sugarcane leaves, banana leaves, peanut hull, cotton wastes, cocoa shells, paper wastes and saw dusts (Cangy and Peerally, 1995 and Jonathan, 2002). In Asia paddy straw waste is used widely for mushroom cultivation while, nowadays saw dust is used in South East Asian countries (Thomas *et al.*, 2002).

The mushroom substrates after its usage in production of mushroom is called after many names like, Spent Mushroom Waste (SMW), Spent Mushroom Compost (SMC) (Fasidi *et al.*, 2008). SMWs can be derived from many different mushroom substrates (Abena *et al.*, 2015, Gashaw *et al.*, 2020, Menkissoglou-Spiroudi *et al.*, 2014, Sbhatu *et al.*, 2019, Sendi *et al.*, 2013 and Tavarwisa *et al.*, 2021) with the potential for reuse in either mushroom production or in farming land as organic fertilizers. About 5 Kg of SMW are produced for each kilogram of Mushroom (Williams *et al.*, 2001). An estimated 10–50 million metric tonnes of spent mushroom compost are expected to be generated annually worldwide (Aderemi *et al.*, 2014, Gonani *et al.*, 2011, Lau *et al.*, 2003, Philippoussis *et al.*, 2004, Odamtten *et al.*, 2016 and Williams *et al.*, 2001). The bulkiness of SMW makes disposal more laborious. Unregulated disposal of SMS causes land, water, and air pollution together with the nuisance in the surroundings (Ahlawat and Sagar, 2007). Nowadays various studies on using it in the field of organic manure, increasing the microbial population, or a substitute for peat are been on the way.

SMWs are naturally non-toxic to plants and so, they are used as soil amendment for different crops (Fasidi *et al.*, 2008). Various research has proved that usage of SMW is beneficial and it provides micronutrients, and it also helps in improving soil water holding capacity, soil aeration (Kadiri and Mustapha, 2010) and also prevents soil hardening. It is also used as a potting

soil mixes in horticulture. Most of the potting materials like peat moss or forest humus used to provide a nutritive soil for the flowering plants. Presently SMC are used instead of that which indirectly reduces the pollution level created by the substrates, in turn is useful by turning it into an environmentally friendly organic manure, which helps in utilization of natural resource.

Apart from that SMW can be used as medium for vermiculture, in agriculture and also for bioremediation of contaminated soils. (Danny, 2002). SMC has a low ratio of C:N ratio which can makes it a good fertilizer, with lots of hyphal protein because of the decomposition of cellulose and lignin of the substrate. To improve soil quality of an alkaline soil SMW can also be used as an acid fertilizer. SMW contains rich and valuable organic materials, which makes it convenient for recycling in different forms (Danny, 1992 and Szmidt and Convay, 1995). Spent substrate from *Agaricus bisporus* has been evaluated for treatment of acid mine drainage [24] and textile dyes [25]. The spent substrate of various *Pleurotus* species has been investigated for the removal of nickel-contaminated water (Tay et al., 2011) and copper (Tay et al., 2010) and antibiotics in waste water from swine (Chang et al., 2014), pesticides in effluents from fruit packing industry (Karas et al., 2015), and textile dyes (Singh et al., 2011). Some mushroom species have been investigated for fluoride removal from drinking water (Chen et al., 2015), methylene blue from waste industrial waters (Yan and Wang, 2013 and Yan et al., 2015), and sulfa antibiotics (Zhou et al., 2016). The main reason for the use of SMSs as a fertilizer is because they are rich in calcium, nitrogen, ash, and protein (Lou et al., 2017 and Lao et al., 2015). Thus, the reuse of SMSs for soil conditioning has turn out to be the focus of consideration as it is also abundant in nitrogen. Besides, it is rich in vital nutrients for plant growth and organic materials that make it a suitable material for soil mulching and improvement. SMS is rich in phosphorus and is always applied to agriculture land to enhance soil organic matter and nutrient contents (Zhu et al., 2012).

Nowadays ways of decreasing the usage of chemical fertilizers are being studied. In order to reduce chemical fertilizer, organic fertilizers are used at various combinations, which indirectly reduces the environmental pollution and directly increases the yield. Application of SMC could also increase the activity of soil microorganisms, and Earthworms. Spent mushroom substrates are rich sources of xenobiotic degrading bacteria, actinomycetes and lignolytic fungi. These microorganisms degrade pollutants to innocuous compounds such as carbon dioxide and water. These microorganisms can bio transform pollutants into less toxic substances and or lockup

pollutants with the organic matrixes thereby reducing pollutants bioavailability (Semple *et al.*, 2001). SMS are rich in plant growth promoting rhizobacteria (PGPR) that colonize the roots of plants to enhance plant growth and reduce diseases, nematodes or insect damage (Reddy *et al.*, 2013).

Azospirillum

Azospirillum.spp is a known example for plant growth promoting rhizobacteria (PGPR) (Kloepper 1992) that could be used as inoculants in Horticultural production. The occurrence of *Azospirillum* species is widespread in the environment including tropical, subtropical, temperate regions. Apart from being a general plant colonizer (Bashan *et al.*, 2004) it is remarkably versatile, it is not only able to fix atmospheric nitrogen (Dobereiner and Day 1976) but also to mineralize the nutrients from the soil to sequester Fe, to survive the environmental conditions which is harmful in nature. In addition, it helps to survive the negative effects of abiotic stresses. Under certain environmental and soil conditions, *Azospirillum* could positively influence plant growth, crop yield and N content. It includes some other biological functions such as auxin production.

Other than *Azospirillum* some other species such as phosphobacteria also plays a vital role in the crop yield and improvement of plants. Generally, Phosphorus is one of the major elements necessary for the plant growth like other elements, it plays a vital role in plant growth the ability of soil microorganisms to solubilize phosphate is an important trait in plant growth to increased yield and smaller use of fertilizers. These microorganisms and mushroom spent produces a high yield of growth of plants. which is qualitatively and quantitatively high as well as which is organic in nature, pesticide free chemical free and ecofriendly whereas the role of organic manure is very well seen in greens which actually Fruits and vegetables are universally promoted a content of nearly and nutrients eventually fruits and vegetable supply dietary fiber, vitamins and minerals. They are also a source of phytochemicals which actually function as antioxidant, phytoestrogens and anti-inflammatory agent.

Azospirillum is a gram negative, microaerophilic, non-fermentative, and nitrogen fixing bacterial genus. This free living rhizobacteria is included in the alpha subclass of proteobacteria belonging to Rrna Subfamily (Xia *et al.*, 1994) encompassing ten species. It has been one of the most studied plant growth-promoting bacteria (PGPB) since its discovery by Martinus Beijerinck in the Netherlands in 1925, these two characteristics have been considered the cornerstone of the

effect of this genus on plant growth and crops because *Azospirillum* is one the most studied PGPB worldwide.

Azospirillum strains is considered to be versatile because it has been identified, isolated, from agricultural soils as well as habitat as diverse as contaminated soils, fermented products, sulfide springs, and microbial fuel cells (Reis *et al.*, 2015; Anandham *et al.*, 2019 and Tikhonova *et al.*, 2019). It is also found to be distributes worldwide and different strains and species have been isolated from several countries, including Argentina, Brasil, China, Taiwan, Korea, Russia, Pakistan, and Iraq. *Azospirillum* plant interactions have been introduced a wide array of mechanism to demonstrate the beneficial impacts of this bacterium on plant growth. Major changes in the plant root architecture is the main outcome of inoculation with *Azospirillum*. It is generally accepted that these developmental responses are triggered by the production of bacterial phytohormones, and more specifically by the biosynthesis of indole-3-acetic acid (IAA) (Cassan *et al.*, 2014). Despite exhaustive efforts to define a single mode of action to explain the plant growth facilitated by inoculation with *Azospirillum* the mode is still undefined however some hypotheses have been proposed to better understand the benefits of the *Azospirillum*-plant interaction (Bashan *et al.*, 2004 and Bashan and De-bashan 2010. *Azospirillum* have a versatile display of C-N metabolism which help them to make a competitive environment of the rhizosphere which is well adapted.

Azospirillum was found to have a positive effect on plant growth due to Nitrogen fixation. And this was emphasized by Dobereiner *et al.*, (1970) and Oken *et al.*, (1983) as significant increase in total Nitrogen in shoots and grains in plants was evaluated using acetylene reduction assay. *Azospirillum* spp. modes of action were initially explained by the additive hypothesis where the effects of small mechanisms operating either at the same time or consecutively create a larger final effect on plants (Bashan and Levanony 1990). *Azospirillum* spp. have been associated to several mechanisms to promote plant growth and a wide range of studies have detailed the beneficial effects of inoculation with these rhizobacteria. the improvement of plant growth by *Azospirillum* spp. has been mostly attributed to their capacity to fix atmospheric Nitrogen and to produce phytohormones; it is less attributed to the bio-disposition of nutrients, expression of enzymes, synthesis of compounds related to plant stress mitigation, and competition against phytopathogens,

among other mechanisms. However, taken individually, none of these mechanisms has been found to be fully responsible for the changes observed in inoculated plants (Bashan and Debashan, 2010).

Azospirillum stimulates the plant root growth by inducing an increase in the water absorption and nutrient acquisition rates (including nitrogen), which improves the assimilation of nitrogen in the biomass and in turn plant growth. This capacity would be mediated by the bacterial colonization of the roots and/or their ability to produce different phytohormones, mostly during early stages of plant development. Consequently, the increase in the root biomass would increase the supply of root exudates into the rhizosphere, which would increase the bacterial population associated with the roots and improve their ability to colonize this organ and the rest of the plant. The combined inoculation of legumes with rhizobia and *Azospirilla*, defined as co-inoculation, could improve plant performance due to the complementary nature of the mechanisms of both bacteria. In soybean crops, co-inoculation resulted in both early initiation of nodule ontogenesis and an increase in the number of nodules, thereby increasing the concentration of nitrogen in the shoots and improving the plant growth, particularly under drought conditions (Chibeba *et al.*, 2015 and Cerezini *et al.*, 2016).

Azospirillum possesses the potential to solve other environmental problems, such as preventing soil erosion by improving the growth of plants on barren and degraded lands that have lost their capacity to support regeneration, and participating in phytoremediation strategies to decontaminate soils, all leading to healthier environments (de-Bashan *et al.*, 2012). Although these uses are not yet widespread Puente and Bashan, (1993) demonstrated that *A. brasilense* inoculated on the cardon cactus (*Pachycereus pringlei*), the world's largest cactus that stabilizes topsoil in its usual scrub habitat in the Sonoran Desert (Mexico), improves plant growth characteristics.

Phosphobacteria

Phosphorus, is one of the essential macronutrients for all living organisms, which is required in large amounts for the growth and development of plants. Phosphorus in total soil is high, but still its bioavailability to plants is suboptimal in most of the soils because of high fixation rates into inorganic and organic insoluble complexes. In this regard, the rhizospheric microbiota plays a key role in the facilitation of phosphorus nutrition. These are recognized long back as an environmental-friendly alternative for using chemical phosphorus. Though phosphorous is found to be around 0.2% of a plants dry weight, its requirement for a plants growth and development is

high. Phosphorous is an essential component as it is present in Nucleic acids, ATP and also in phospholipids. And also, inorganic phosphorus regulates several important enzyme reactions and metabolic pathways (Schachtman *et al.*, 1998). Thus, low phosphorus availability represents a stressful condition for plants and imposes severe crop yield limitations (Bononi *et al.*, 2020).

Even when the total amount of phosphorus in soils is usually high (between 400 and 1,200 mg kg⁻¹ of soil), its bioavailability to plants is less than 1% (Rodríguez and Fraga, 1999 and Zhu *et al.*, 2018). Inorganic phosphorus availability for plants is low because of its immobilisation in phosphorus-insoluble complexes mainly with iron, aluminium and calcium, a phenomenon that strongly depends on soil pH. Besides, soils can contain organic phosphorus forms that cannot be easily assimilated by plants and also form complexes (Rodríguez and Fraga, 1999).

Plants interact with deleterious, neutral and beneficial microorganisms during its life cycle (Tilak *et al.*, 2005). Beneficial microorganisms include a wide range of bacteria and fungi. Some of them establish sophisticated mutualistic symbioses involving the formation of specialized symbiotic structures on plant hosts. This is the case of rhizobial and actinorhizal bacteria and mycorrhizal fungi. On its part, multiple free-living microorganisms that colonise the rhizosphere and/or endophytically invade plant tissues exert a variety of beneficial effects on plant hosts. Collectively, microbes that exhibit the above-mentioned features are designated as plant growth-promoting microorganisms (PGPM). These microorganisms can affect plant growth either directly or indirectly by several different mechanisms (Ali and Glick, 2019). In particular, phosphobacteria are an important subgroup of PGPM capable of providing plants with available phosphorus and promoting plant growth (Jorquera *et al.*, 2008).

Phosphobacteria are the bacteria that helps in solubilizing and mineralizing phosphorus present in the soil (Widdig *et al.*, 2019). Research interest in phosphobacteria has been mainly focused on PSB because of their well-proven effect on plant growth and soil fertility (Liu *et al.*, 2020) in addition to inorganic phosphates, a large fraction of total soil P is present in organic form (Balaban *et al.*, 2017). Although organic phosphorus accounts from 30% to 65% of total soil phosphorus, they are usually not accessible for plants (Richardson, 2001). Frequently, a high proportion of organic phosphorus in soil is represented by phytate (myo-inositol phosphate) and phytate-metal complexes (Balaban *et al.*, 2017). As opposed to plants, some soil phosphobacteria

produce phosphohydrolases, known as phytases, a particular class of phosphatases able to mineralise organic phosphorus from phytate and related organic phosphate sources, thus playing a central role in soil (Singh *et al.*, 2020).

Azospirillum inoculation has improved the negative effects of abiotic stress on crops. This has attracted the researchers interested in developing field applied studies (Okon and Labandera-Gonzalez, 1994). Okon *et al.*, (2015) the diverse modes of action of *Azospirillum* mostly stimulate plant root growth, inoculation with this microbe could contribute to the increase and stabilization of crop production. The greatest contribution of *Azospirillum* inoculation to grain yield was observed in winter cereals followed by summer cereals and other crops *Azospirillum* showed variable results and a multiplicity of interactions related not only to crop management practices but also to environmental conditions.

Vegetables which require marginal processing and are consumed fresh have gained importance and attention in the international vegetable market (Naujokat *et al.*, 2004). The increase in demand for baby leaf greens has resulted in many farmers gearing their production towards baby greens such as baby lettuce, baby spinach, and Swiss chard (Vernieri *et al.*, 2004).

Spinacia oleracea

According to nutritionist and leafy vegetables are important in our weekly food intake. *Spinacia* is a nutrient rich important leafy vegetable. *Spinacia oleracea* L. (Palak) belonging to the family Chenopodiaceae, is native to Central Asia, most probably Persia (Asia) and has been grown in China, since at least the 7th century and was used in Europe, since at least the 13th century (Cai *et al.*, 2017 and Sabaghnia *et al.*, 2016). It is an annual plant grown in both spring and autumn. It is a leafy cool-season vegetable with global cultivation usually consumed after boiling either fresh or frozen leaves or raw consumed in salad (Alessa *et al.*, 2017, Eriksen *et al.*, 2016 and Yoon *et al.*, 2017). Spinach is an annual plant species and the closely related species, *S. tetrandra*, is considered to be its ancestor, and other related species comprise *S. spinosa*, *S. inermis* and *S. turkestanica*. The classification of numerous spinach cultivars is based on seed form (round or prickly); leaf texture (smooth or crinkled); leaf colour, shape and pose, and petiole length (AlKhayri, 2012). The edible leaves are arranged in a rosette, from which a seed stalk emerges. The simple leaves are somewhat triangular or ovate and may be flat or puckered. The flowers are inconspicuous and produce small dry fruits (Hassandokht, 2012).

The nutritional value of fresh spinach, with 91% of moisture content, shows between 0.4 % and 0.6 % lipid content, around 2.9% protein content, and contains good levels of essential amino acids, except sulfurous amino acids (methionine) and tryptophan. Although the carbohydrate content is very low (2-10%), the fiber content is high (2.2%) (Murica *et al.*, 2020). Palak is good source of iron and is packed with Vitamin C, vitamin A, vitamin E and minerals like magnesium, manganese, calcium and folic acid. It is also rich in carotenoids, beta-carotenoids and lutein. It is also a good source of bioflavonoid quercetin with many other flavonoids which exhibits anti-oxidant, antiproliferative, anti-inflammatory, antihistaminic, CNS depressant, protection against gamma radiation, hepato protective along with many other benefits (Subhash *et al.*, 2010). It is also designated as 'nature anti-aging wonders' which has various medicinal values (Gupta and Prakash, 2009). It is also a good source of chlorophyll, which is known to aid in digestion (Roughani *et al.*, 2011, Gaikwad *et al.*, 2010 and Verma, 2018).

The leaves of spinach are traditionally used in various folklore medicine as cooling, emollient, wholesome, antipyretic, diuretic, maturant, laxative, digestible, anthelmintic, urinary calculi, 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, arrest vomiting, biliousness, flatulence and febrile (Metha and Belemkar, 2014 and Verma, 2018). Ameliorative effects of spinach seeds on carbon tetrachloride induced hepatotoxicity. The in vitro and in vivo hepatoprotective effects of spinach seeds were examined and suggests that these seeds acts as therapeutic agent in liver diseases. The seeds are useful in fevers, leucorrhoea, urinary discharges, lumbago, brain and heart diseases. Seeds are laxative and cooling (Rao *et al.*, 2015 and Verma, 2018).

Spinach consumption is recognized to have wonderful outcomes on human fitness and has been linked to decreased risk of most degenerative illness associated with growing older (Williamson, 1996), such as coronary heart ailment (Liu *et al.*, 2000), cardiovascular disorder (Gandini *et al.*, 2000), Alzheimer's disorder (Liu, 2001), cataracts (Joshi *et al.*, 2001), and several forms of most cancers (Kang, 2005). It is used as a dietary vegetable and is available all over the world. Therefore, it is worthwhile to conduct detailed studies in order to explore the full potential of this plant in reducing inflammation in humans from the point of view of cost and availability for people at all socioeconomic levels (Garg *et al.*, 2010 and Gupta *et al.*, 2018). Production of spinach is still limited with low yields being characterized by poor agronomic

practices including soil nutrient management options. Low yields of spinach and the problem of bitter taste caused by costly inorganic fertilizers thus require the intervention of using organic fertilizers such as spent mushroom substrate (SMS) which itself also has challenges with disposal.

Materials and Methods

Materials and Methods

A pot culture study was carried to find out the effectiveness of Spent Mushroom Waste and Azospirillum usage as organic fertilizer.

3.1 Pot Culture Experiment

1. Control - Soil is taken in grow bag. Without any other addition of organic waste or bio-inoculums, it is used as control. It is taken in triplicates. SMW is dried well.
2. Soil + SMW - Soil along with Spent Mushroom Waste (SMW- 1 Kg dried) was well mixed with soil and taken in growbags.
3. Soil + AS+AP - Soil along with Azospirillum + Azophosphate (50 g each) was added at regular intervals in growbags.
4. The above set of treatments were carried out as triplicates with coco peat (2 kg) mixed along with the potting soil.

3.2 Plant material

1. Palak (*Spinacia olerace*) green leaves were used as experimental plant
2. Soil mixture with cocopeat is watered well and then seeds (15 seeds) were spread above which a layer of dry soil is added and water is sprinkled on it.

3.3 Physical properties

Physical properties of soil like pH and salinity were measured using pH meter at an interval of 15 days and 30 days after germination of seeds to evaluate the changes in the soil properties due to SMW or bio-inoculums.

3.4. Biochemical properties

3.4.1 Estimation of Chlorophyll and Nitrogen using nutrient analyser

Fresh leaves were taken to read values for chlorophyll content and nitrogen present in all the leaf samples using Panomex Plant Nutrient Analyser. Triplicate values were taken for further Statistical Analysis.

3.4.2 Total Carbohydrate (Hedge and Hofreiter, 1962)

Principle

Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone, a green coloured product with an absorption maximum at 630nm.

Materials

- 2.5N HCl
- Anthrone reagent: Dissolve 200 mg anthrone in 100 ml of ice cold 95 % H₂SO₄ prepared fresh before use.

Standard glucose (Stock): Dissolved 100 mg in 100 ml of water.

Working standard – 10 ml of a stock solution was diluted to 100 ml distilled water. After adding few drops of toluene stored in refrigerator after adding a few drops of toluene.

Procedure

- About 100 mg of the sample was taken in a boiling tube and it was hydrolysed by keeping it in a boiling water bath for three hours with 5ml of 2.5N HCl and cooled to room temperature.
- Then it was neutralized with solid sodium carbonate until the effervescence created.
- The volume was made up to 100 ml and centrifuged.
- The supernatant was collected and 0.5 and 1 ml aliquots were taken for analysis.
- The standard was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard and '0' served as blank.
- The volume was made up to 1 ml in all the tubes including the sample test tubes by adding distilled water.
- Then, 4 ml of anthrone reagent was added and heated for eight minutes in a boiling water bath.
- Then it was cooled rapidly and the green colour developed was read at 630 nm.
- A standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis.
- From the graph, the amount of carbohydrates present in the sample tube was calculated.

Calculation

Amount of carbohydrates present in 100mg of the sample is calculated by

$$\frac{\text{mg of glucose}}{\text{volume of test sample}} \times 100$$

3.4.3 Estimation of protein (Lowry *et al.*, 1951)

Principle

The blue colour developed by phosphomolybdic phosphotungstic components in the Folin-Ciocalteu reagent by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartarate are measured in the Lowry's method.

Materials:

- Reagent A - 2 % sodium carbonate in 0.1 sodium hydroxide
- Reagent B - 0.5 % copper sulphate (CuSO₄.5H₂O) in 1% potassium sodium tartarate.
- Reagent C - Alkaline copper solution : Mixed 50 ml of A and 1 ml of B prior to use.
- Reagent D - Folin-Ciocalteu Reagent.

Stock standard : 50 mg of bovine serum albumin (Fraction V) was weighed and dissolved in distilled water and the volume was made up to 50 ml in a standard flask.

Working standard: About 10ml of the stock solution was diluted to 50 ml with distilled water in a standard flask. One ml of this solution contain 200 µg proteins.

Procedure

Extraction of protein from sample

Extraction is usually carried out with buffers used for the enzyme assay. About 50mg of the weighed sample was ground well with pestle and mortar in 5- 10 ml of the buffered centrifuged. The sample was used for protein estimation.

Estimation of protein

- About 0.2, 0.4, 0.6, 0.8, and 1 ml of working standard were pipette into a series of test tubes and 0.1 ml and 0.2 ml of the sample extract in two other test tubes.
- The volume was made up to 1 ml in all test tubes. A tube with 1 ml of water severed as the blank.
- About 5 ml of reagent C was added to each tube including the blank, mixed well and allowed to stand for 10 minutes.

- Then 0.5 ml of reagent D was added. Mixed well and incubated at room temperature in the dark for 30 min. Blue colour developed was read at 660 nm.
- A standard graph was drawn and the amount of protein present in the sample was calculated.

Calculation

The amount of protein present in the sample was expressed in

$$\text{mg/g or 100g} = \frac{\text{mg of protein}}{\text{volume of the test standard}} \times \text{concentration of the standard}$$

Results & Discussion

Results and Discussion

Mushroom cultivation which is a profitable agroindustry leaves behind Spent Mushroom Waste (SMW) as agrowaste. Concerning the disposal of SMW, this study is carried to evaluate the potential of its reuse in production of leafy vegetable *Spinacia oleracea*.

4.1 Physical Parameters

Table. 1: pH and Salinity in soil after 30 days of plant growth

	pH		Salinity (ppm)	
	0 th Day	30 th Day	0 th Day	30 th Day
Control	6.37	6.73	116.8	114.4
SMW	6.37	7.39	116.8	23.6
AZ+AP	6.37	7.07	116.8	66.5

According to Fage, (2003) high pH and Electric conductivity leads to increase in the level of nutrients during composting. The pH of the soil from 0th day to 30th day shows increase, and got increased by 1.02 units and 0.71 units in SMW and Azospirillum treated soil respectively. According to Jin, (2020) SMS (Spent mushroom substrate) application increased soil pH by 0.61 units compared with non-amendment Cd-contaminated soil.

Application of SMW has helped decreasing the salinity of the soil by 93.2 units and 50.3 units in SMW and Azospirillum treated soil respectively.

4.2. Biochemical Parameters

4.2.1 Chlorophyll and Nitrogen Analysis

Nutrient analyser is used to read the chlorophyll (SPAD) and nitrogen (mg/g) content in the leafs of *Spinacia* under various soil treatments. The SPAD (Silicon Photon Activated Diode) meter provides a very easy, swift, and nondestructive method for estimating relative leaf chlorophyll content. SPAD value is proportional to the amount of chlorophyll present in leaf, and a linear relationship exists between SPAD value and leaf nitrogen concentration (Sendi *et al.*, 2013).

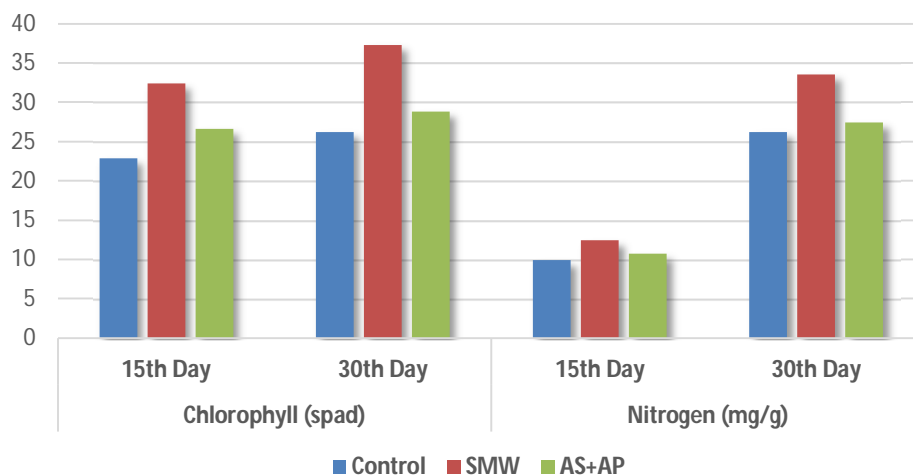
Table. 2: Chlorophyll and Nitrofen content in the leaf samples

	Chlorophyll (SPAD)		Nitrogen (mg/g)	
	15 th Day	30 th Day	15 th Day	30 th Day
Control	22.9 ± 0.43	26.2 ± 0.39	9.9 ± 0.37	26.2 ± 0.47
SMW	32.4 ± 0.33	37.3 ± 0.51	12.4 ± 0.40	33.5 ± 0.26
AS+AP	26.6 ± 0.49	28.8 ± 0.40	10.7 ± 0.52	27.4 ± 0.60
SEd	0.3724		0.3912	
CD (p<0.05)	0.5289*		0.6162*	

SPAD = Soil Plant Analysis Development

Values are mean ± SD of three samples in each group

* - Significant at 5% level (p<0.05)

Fig 1: Comparison of Chlorophyll and Nitrogen content in *Spinacia* under various treatment of organic fertilizers

SPAD value of 15 days leaf grown with SMW showed higher value while leaf taken from control (without any fertilizer) had a low value of 22.9 and 32.4 respectively and the same trend was seen in 30 days of leaf samples too. Higher SPAD values indicate greener and healthier plants. Altland *et al.* (2002) also confirmed that leaf chlorophyll content is often correlated with the nitrogen content of growth media.

4.2.2 Total Carbohydrate

Carbohydrates present in the leaves of *Spinacia* was estimated in control and other two treatments. Level of total carbohydrate present in the leaf was found to be at maximum in SMW treated soil (43.89 mg/g) and minimum in control without any soil supplements (37.02 mg/g).

4.2.3 Protein

Protein estimation was done in the leaves of *Spinacia*. Protein present in SMW (21.98 mg/g) is higher than the protein synthesized and stored in leaves of Azospirillum (18.33 mg/g)

Table, 3: Carbohydrate and Protein content in 30 days of leaf sample from various treatments

	Carbohydrate	Protein
Control	37.02 ± 1.005	16.11 ± 1.981
SMW	43.89 ± 0.678	21.98 ± 2.312
AS+AP	38.93 ± 2.210	18.33 ± 0.782
SEd	0.9742	0.8113
CD (p<0.05)	0.0871	0.0752

Values are mean ± SD of three samples in each group

* - Significant at 5% level (p<0.05)

Fig. 2: Graphical comparison of carbohydrate and protein present in various treatments of organic fertilizer at 30 days

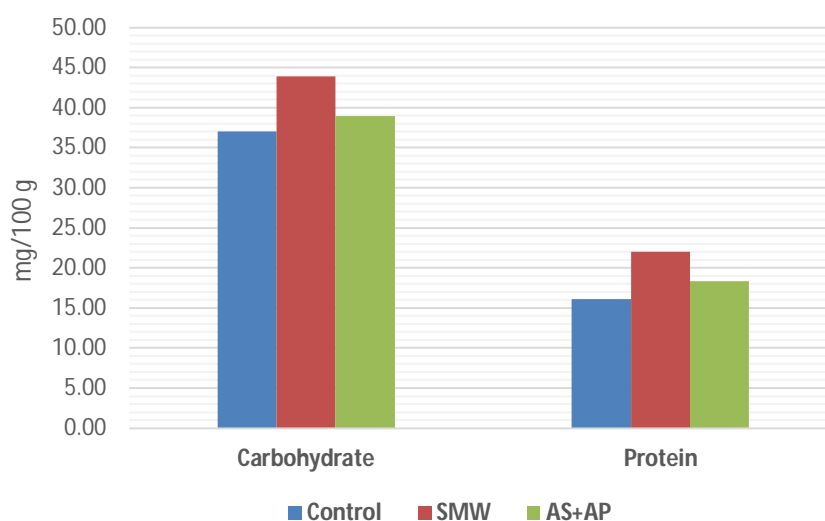


Plate 1: Growth of *Spinacia oleracea***Plate 2: 15th Day growth in Grow Bag****4.3 Elemental analysis**

Energy dispersive X-ray analysis (EDAX) showed the presence of C, O, Mg, Al and Si in Soil (15th day) without any amendment of organic fertilizers. Same elements were found in soil (30th day) with amendment of spent mushroom waste, where the soil carbon was found to be in increase than other elements. Azospirillum amended soil also had the same improvement in soil.

The soil organic carbon was evidently seen to be increase in Azospirillum amended soil, than SMW treated soil. Similarly, Gumus and Seker, (2017) also stated that spent mushroom waste has increased the soil organic carbon than the other treatments which they have used for their study.

SMW is an effective soil amendment and conditioner, and addition of SMW has been found to considerably increase yield of many crops. In our research findings total carbohydrates and protein were high in plants grown in SMW amendment soil.

Table 4: List of elements present in the soil on 15th day after growing *Spinacia*

Control		AZ+AP		SMW	
Element	Weight %	Element	Weight %	Element	Weight %
C K	30.34	C K	41.32	C K	28.41
O K	51.68	O K	45.80	O K	44.28
Mg K	1.15	Mg K	1.14	Mg K	0.96
Al K	6.83	Al K	4.87	Br L	8.73
Si K	10.00	Si K	6.87	Si K	14.62
				K K	1.75
				Ca K	1.25

Table 5: List of elements present in the soil on 30th day after growing *Spinacia*

Element	Weight %	Element	Weight %	Element	Weight %
C K	32.48	C K	40.76	C K	32.87
O K	50.83	O K	45.37	O K	48.08
Mg K	0.91	Mg K	0.80	Na K	0.85
Si K	6.93	Br L	6.34	Mg K	0.87
Cl K	1.00	Si K	6.73	Al K	3.20
K K	0.88			Si K	5.36
Sn L	4.37			Mo L	0.77
Ca K	0.48			K K	0.54
Fe K	1.62			Sn L	2.29
Co K	0.51			Ca K	0.61

Fig.3: Graphical representation of elements present in the soil on 15th day after growing *Spinacia*

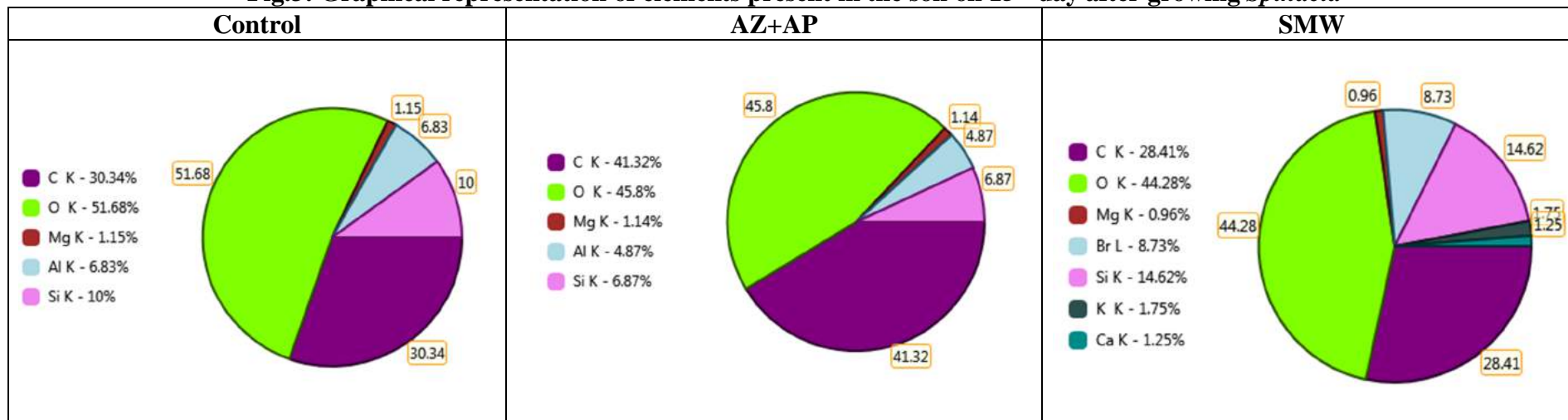


Fig.4: Graphical representation of elements present in the soil on 30th day after growing *Spinacia*

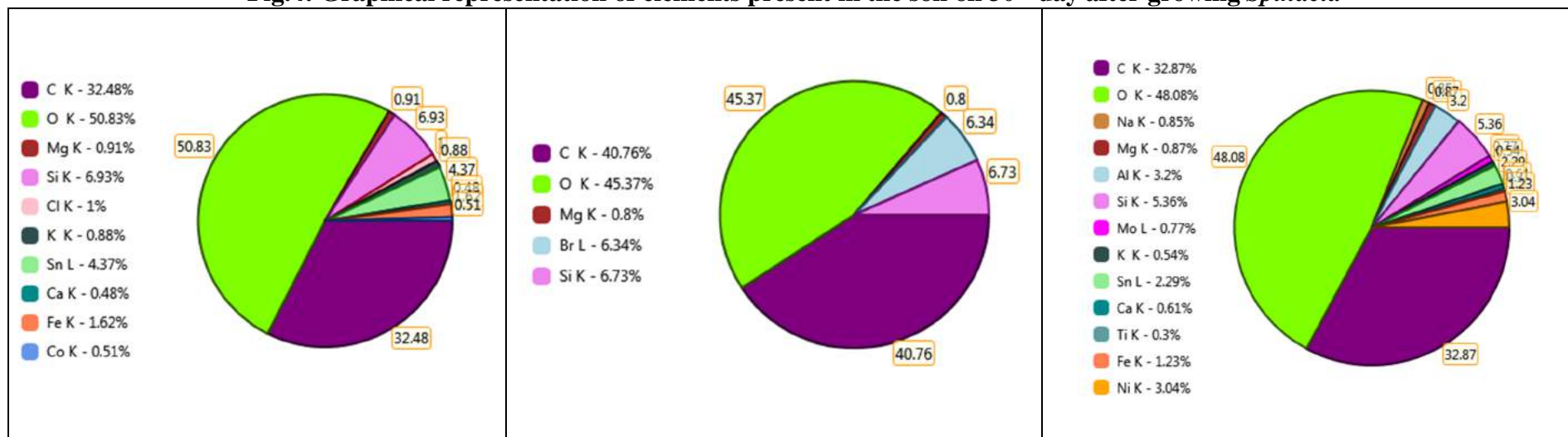


Fig.5: EDAX Spectrum Results of soil (control) on 15th day of growing *Spinacia*

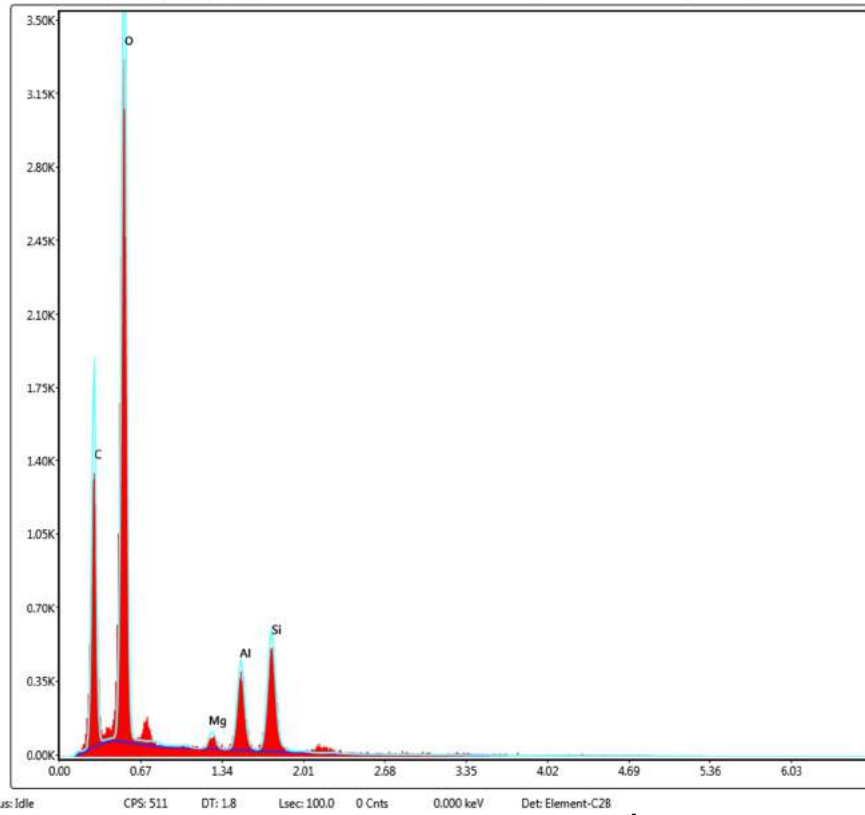


Fig.6: EDAX Spectrum Results of soil (control) on 30th day of growing *Spinacia*

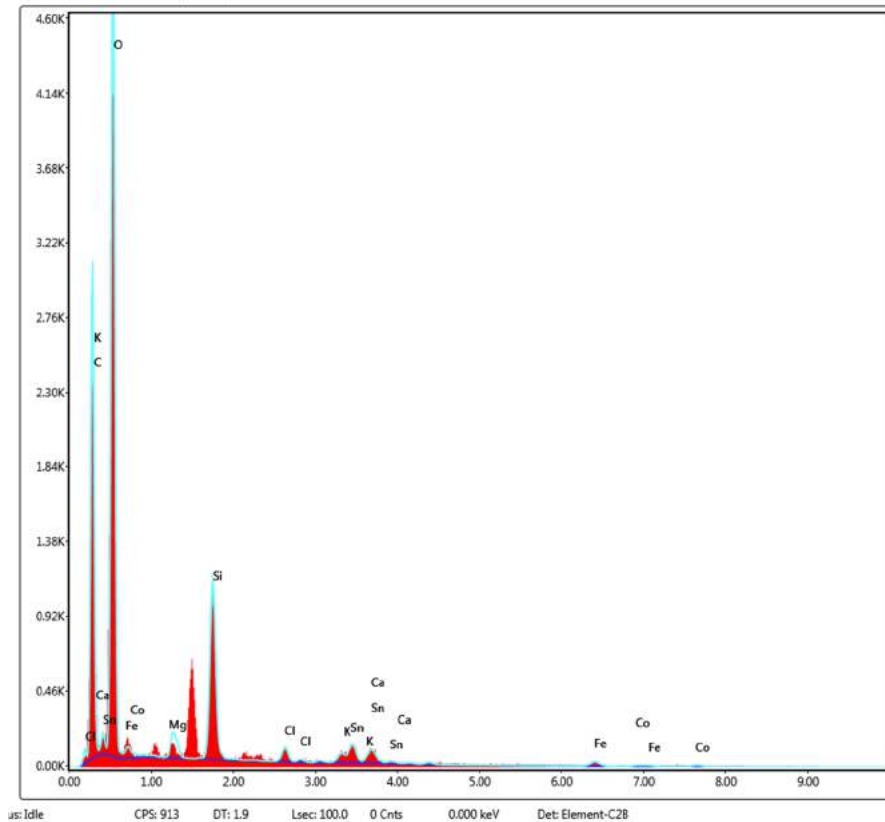


Fig.7: EDAX Spectrum Results of soil (SMW) on 15th day of growing *Spinacia*

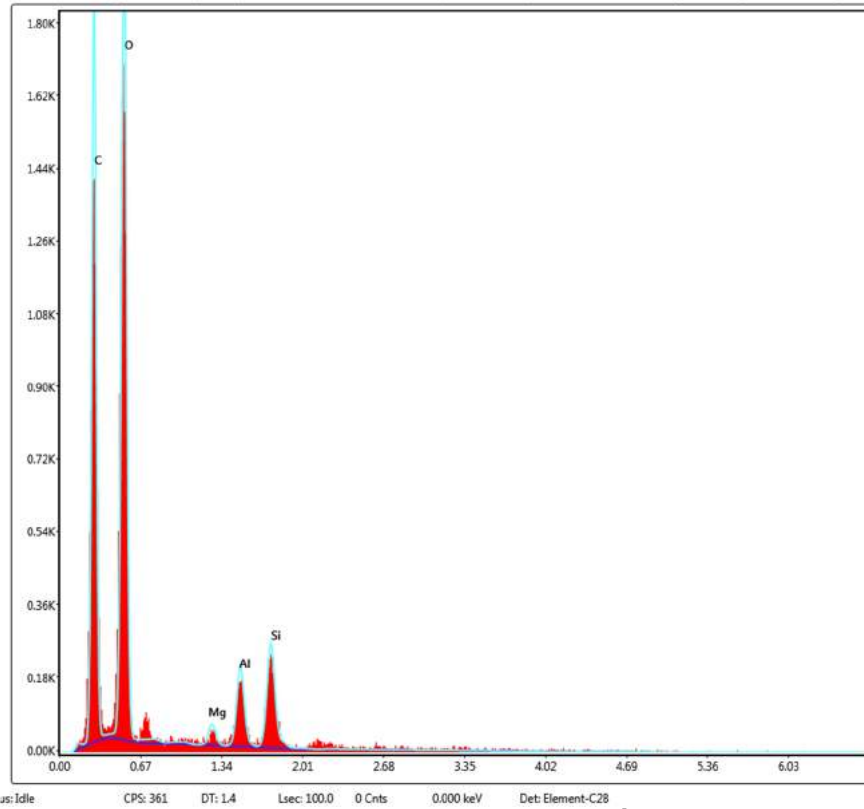


Fig.8: EDAX Spectrum Results of soil (SMW) on 30th day of growing *Spinacia*

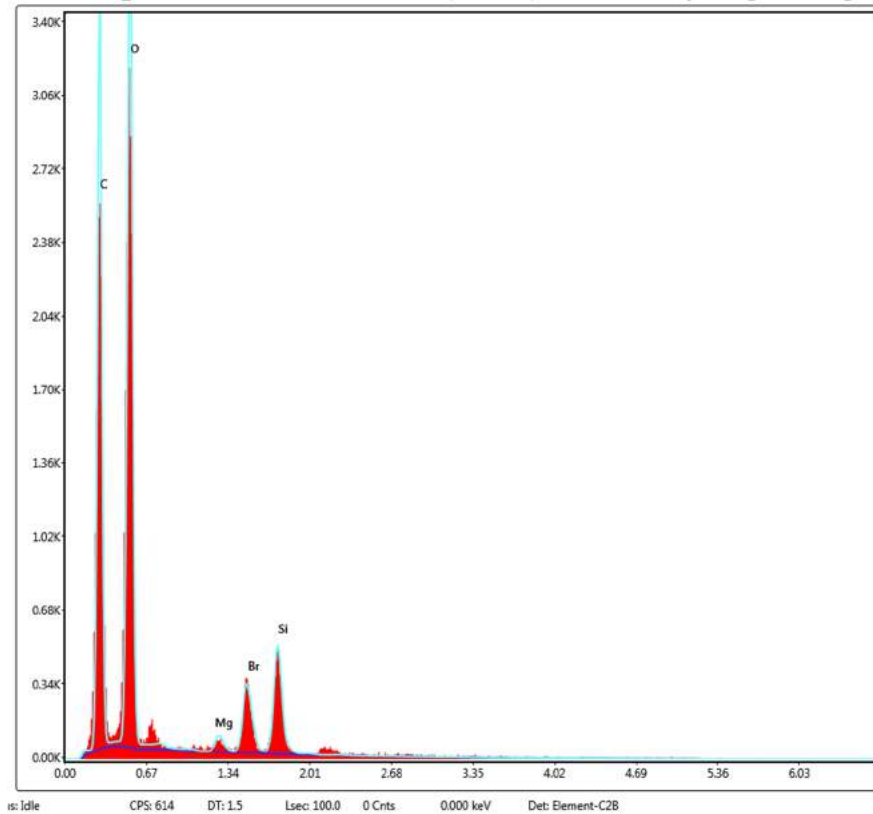


Fig.9: EDAX Spectrum Results of soil (AZ+AP) on 15th day of growing *Spinacia*

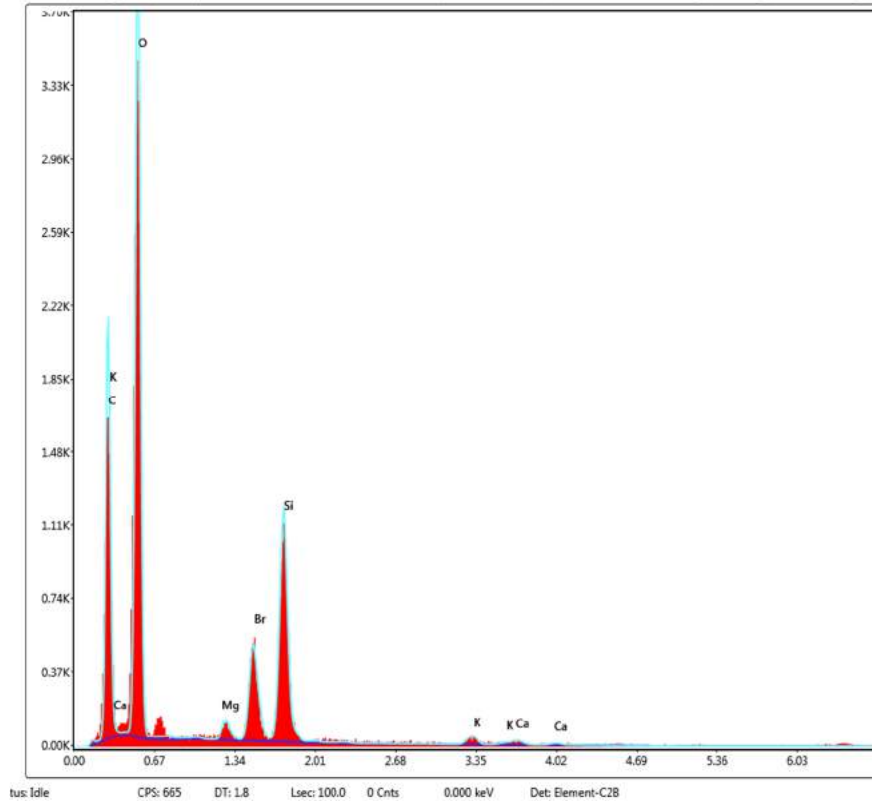
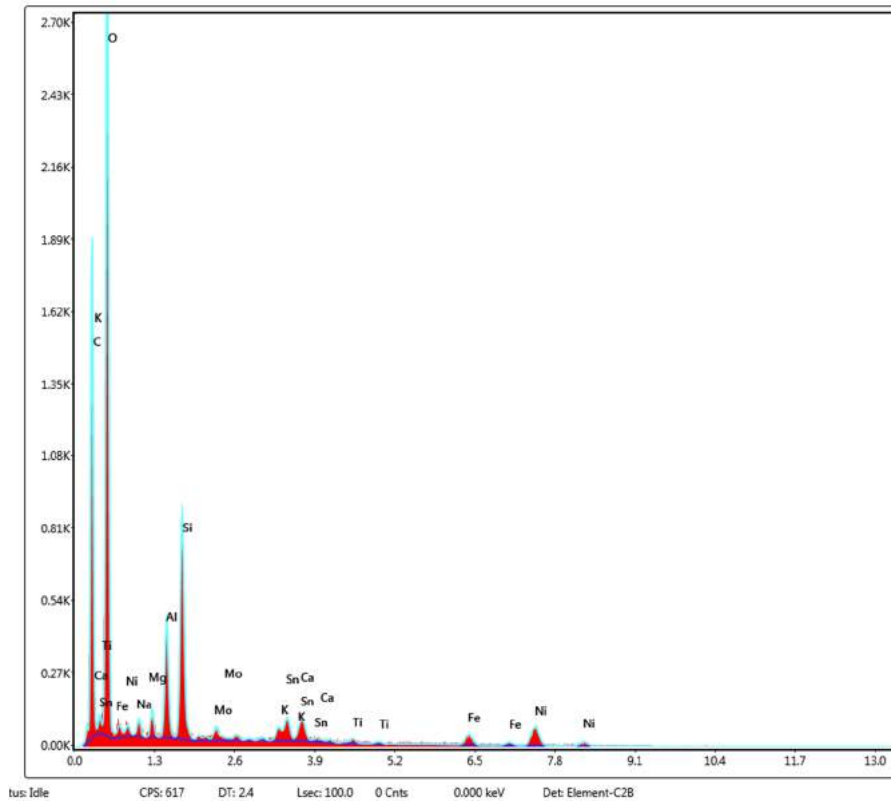


Fig.10: EDAX Spectrum Results of soil (AZ+AP) on 30th day of growing *Spinacia*



Iwase *et al.* [Iwase *et al.*, 2000] observed that addition of SMW to soil increased the yield of tomato 7-fold, and the yield of lettuce and radish 2-fold each. A positive impact of using SMW as a growing substrate component on the productivity and quality of different vegetables and other horticultural crops has been confirmed by many researchers worldwide [Polat *et al.*, 2009, Medina *et al.*, 2009, Eudoxie and Alexander, 2011, Jonathan *et al.*, 2011 and Idowu and Kadiri, 2013).

Summary & Conclusion

Summary and Conclusion

The present study is done to compare the effects of Spent Mushroom Waste (SMW) and the bio-inoculum *Azospirillum* in growing *Spinacia olearace*. The use of SMW certainly will help in disposal problems and it also has a positive role as an organic fertilizer.

SMW proves to be very good in improving the pH of the soil. Considerable increase in pH is found in the soil of pot culture where SMW was used to grow the leafy vegetable. This agrowaste can be used to change soil pH condition, if the soil is too acidic. Salinity of the soil was found to be reduced in SMW used soil than others after 30 days. This too proves that SMW helps in reducing the salinity of the soil.

Chlorophyll content was also found to high in both 15th day and 30th day of the leaf grown with SMW as organic fertilizer. An increase in nitrogen content was also recorded in the SMW grown leaf samples of *Spinacia*. Higher the chlorophyll content indicates the greener and healthier the plants are and this is always correlated with the nitrogen content.

Carbohydrate present in the leaf of SMW grown *Spinach* is found to maximum when compared to the leaf sample grown with bio-inoculum and control plants. Protein was also found to be more than other fertilizers used in this study.

The organic carbon in the soil was found to be less in SMW treated soils than other treatments. Other elements present in the soil were analysed by EDAX, which showed that both in the 15th and 30th day the element in the soil increases with the application of SMW. This might be due to the breakdown of complex elements to simpler forms. This also helps plants in absorbing many elements from the soil.

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