

**AKENTEN APPIAH-MENKA UNIVERSITY OF SKILLS  
TRAINING AND ENTREPRENEURIAL DEVELOPMENT**

**PERFORMANCE OF DIFFERENT BIO STIMULANTS ON  
GROWTH AND YIELD OF THREE VARIETIES OF  
LETTUCE**

*(Lactuca sativa)*

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MASTER OF EDUCATION (M.Ed.) IN AGRICULTURE**

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**AKENTEN APPIAH-MENKA UNIVERSITY OF SKILLS TRAINING AND  
ENTREPRENEURIAL DEVELOPMENT**

**MAMPONG - ASHANTI**



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(CROP SCIENCE)**

**SEPTEMBER, 2023**

**DECLARATION**

**STUDENT’S DECLARATION**

I, Benita Akosua Yeboah, declare that this thesis, except quotations and references contained in published works which have all been identified and duly acknowledged, is entirely my original work, and it has not been submitted, either in part or whole, for another degree elsewhere.

SIGNATURE: .....

DATE: .....

**SUPERVISOR’S DECLARATION**

I hereby declare that the preparation and presentation of this work was supervised by the guidelines for supervision of thesis as laid down by the Akenten Appiah-Menkah University of Skills Training and Entrepreneurial Development.

PROFESSOR STEPHEN LARBI-KORANTENG (Principal Supervisor)

SIGNATURE.....

DATE.....

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## LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
Al	Aluminum
Ca	Calcium
CDC	Centers for Disease Control
CGN	Centre for Genetic Resources
EBIC	European bio-stimulants industry council
FAO	Food and Agriculture Organization
FA	Fulvic Acid
Fe	Iron
HPTA	Humic Products Trade Association
HS	Humic substances
I	Iodine
IAA	Indole acetic acid
IHSS	International Humic Substances Society
K	Potassium
Mn	Manganese
Mg	Magnesium
N	Nitrogen
P	Phosphorus
PH	Acidity
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
S	Sulphur
SSR	Simple Sequence Repeat
TRAP	Target Region Amplification Polymorphism
USAD	United State Agriculture Department
VNTRs	variable number tandem repeats
Zn	Zinc

## ABSTRACT

The excessive use of synthetic fertilizers can reduce the nutritional intake of crops and limit plant growth and development. Hence, a study was conducted to assess the impact of different biostimulants on the growth of three varieties of lettuces. The experiment consisted of three types of lettuce (Eden, Omega, and Great Lakes), two types of biostimulants (seaweed with humic and fulvic acid concentration), and a control group. The results showed that applying biostimulants significantly improved growth parameters and dry matter accumulation. The use of fulvic acid with humic acid (FH) and seaweed with humic (SH) significantly enhanced the number of leaves, leaf length, canopy spread, fresh and dry shoot weight, and fresh and dry root weight, compared to the control group. Among all the growth parameters, seaweed with humic produced the highest mean value. The Eden variety had the highest mean value for the number of leaves (11.29), leaf length (8.30), canopy spread (16.26), and dry matter accumulation (4.92), while the Great Lakes had the lowest (0.13). The study revealed that the interaction between the Eden variety and seaweed with humic produced the highest dry matter accumulation, whereas the interaction between the Great Lakes and the control group was the lowest. Regarding the growth parameters, the interaction between the Omega variety and seaweed with humic produced the highest leaf length and canopy, while the interaction between the Eden variety and seaweed with humic produced the highest number of leaves. Therefore, planting the Eden variety of lettuce and applying seaweed with humic biostimulant is a sustainable practice for lettuce production in the Asante Mampong Municipality.

## CHAPTER ONE: INTRODUCTION

### 1.1 Background

In recent years numerous crop producers are paying attention to the use of Bio-stimulant. The European Biostimulant Industry Council (EBIC, 2012) describes biostimulant as ‘Substances and or microorganisms when applied to plants stimulate the natural processes of plants to help in their nutrient uptake, nutrient effectiveness, moderation to abiotic stress and or crop quality independent of its nutrient content’. This process reduces fertilization requirement and support environmental conservation (Bulgari *et al.*, 2015), and also considered widely as an environmentally friendly agrarian practice in plants production (Parađiković *et al.*, 2019). Biostimulants are mainly extracts from raw organic ingredients such as: Humic and amino acids, seaweed extracts, chitin, chitosan and poly and oligosaccharides (Kauffman *et al.*, 2007;Yakhin *et al.*, 2017). Seaweed extracts for instance, have been used in farming long ago as soil conditioners or as plant stimulators, when applied as foliar spray it improves plant growth, photosynthetic activity and resistance to fungi, bacteria and viruses, as well as freezing, drought and salt tolerance, and also improves the yield quality and productivity of many crops (Sharma *et al.*, 2014). Silva *et al.* (2020) added that a combination of seaweed extract, mainly green and brown algae with amino acids and some chemical compounds such as potassium, phosphorous, nitrogen, boron, molybdenum and zinc improve growth and quality, stress tolerance, nutrient accumulation and biosynthesis (Jindo *et al.*, 2020). Parađiković *et al.* (2019) reported that, independent of the type or source, all biostimulants that improve root growth and development of many horticultural plants like vegetables, ornamentals, and medicinal plants.

There are several products available on the market that can be used as biostimulants in various crops (De Pascale *et al.*, 2018). According to du Jardin, (2012), the main categories of biostimulants are based on chitin and chitosan derivatives, humic substances, seaweed extracts, antitranspirants, N-containing substances, and free amino-acids.

## **1.2 Problem Statement**

Over the years, synthetic fertilizers and other chemicals have been used for farming activities which has therefore led to water pollution, land degradation and sometimes poisonous to the farmer. The use of chemical fertilizers is an efficient and low-cost way to supply crops with essential mineral nutrients (Chen, 2006). However, fertilizers can be washed away by runoff (Daverede *et al.*, 2004, Moe *et al.*, 1967) or transformed chemically, physically, or biologically, causing them to become unavailable to crops (Sánchez *et al.*, 2001, Schachtman *et al.*, 1998). As a result, farmers must apply more chemical fertilizer than necessary, and the excess often pollutes the air and water (Vance, 2001). Additionally, the industrial production of chemical fertilizers is an energy-intensive process that significantly contributes to global CO<sub>2</sub> emissions (Vance, 2001). Overuse of these synthetic fertilizers reduces the nutritional quality of the crop and also limits plant growth and development. Organic manures have been used as an alternative to inorganic fertilizers because of their beneficial effects on soil productivity. On the other hand, organic fertilizers, such as compost, sludge, or manure, utilize the nutrients already existing in the agro-ecosystem and require minimal energy input for processing. Moreover, the mineral nutrients embedded in organic materials may be more stable and remain in the soil longer, reducing the likelihood of being washed away or released into the atmosphere (Estavillo *et al.*, 1994, King and Torbert, 2007). However, organic fertilizers possess the downside of not providing crops with readily absorbable, water-soluble nutrients when

required (Chen, 2006). One way to overcome this drawback is by using biostimulants. However, numerous studies on bio stimulant have been undertaken in developed countries on vegetables but little is known about their effects on soils in Ghana and lettuce grown in Asante Mampong.

### **1.3 Justification of the study**

In response to the call to practice sustainable agriculture a new, innovative, environmentally friendly approach such as the application of organic natural plant biostimulants (PBs) in different forms is needed. Biostimulants application to plant crops will help farmers boost crop yield, protect farmlands and waterbodies from poisonous content and help migrate farming systems from synthetic fertilizer to organic forms. Biostimulant enhances crop productivity, fruit set, flowering, plant growth, and nutrient use efficiency, especially under biotic and abiotic stressors (Colla & Rouphael, 2015; Petropoulos, 2020). Lettuce is consumed as salad cooked or uncooked. Application of biostimulants to lettuce varieties will not only guard the environment but protect the nutrient of the lettuce in its fresh or cooked state. Moreover, in lettuce, biostimulants will increase leaf pigments (chlorophyll and carotenoid content) and also enhance the antioxidant potential of the plant.

The increasing demand for crops to feed the growing population is a major concern worldwide. Nutrient requirements for agronomic and horticultural crops must meet acceptable standards to ensure their quality and quantity. Biostimulants have been recognized for their potential benefits, and recent developments have encouraged their use to improve the nutritional efficiency of crops. The availability of nutrients is crucial to the eventual quality and quantity of crops, which are important for both human and livestock

health. However, the land area for growing vegetables is shrinking, and mineral nutrient deficiencies are becoming more common. The use of chemical fertilizers to improve crop yield is being discouraged due to their negative environmental impacts. Biostimulants can provide an effective alternative to chemical fertilization, making them an increasingly attractive option for enhancing crop growth and development.

#### **1.4 Objective of the study**

##### ***1.4.1 Main objective***

The study aimed to assess the performance of different biostimulants on the growth and yield of the three varieties of lettuces grown in the Mampong Municipality.

##### ***1.4.2 Specific objectives***

The specific objectives of the study were to:

1. Determine appropriate lettuce variety suitable for production in the Mampong Municipality
2. Evaluate the performance of different biostimulants on the growth of lettuce.
3. Assess the interaction effects of biostimulants and variety on the growth of lettuce

## CHAPTER TWO: LITERATURE REVIEW

### 2.1 Origin of Lettuce

Lettuce (*Lactuca sativa* L.) belongs to the Asteraceae family (Kim *et al.*, 2016). Lettuce originated in ancient Egypt, where it was cultivated as a weed whose seed was used to make oil. Lettuce was later introduced to Greece and Rome, where it was named *Lactuca* (from which English lettuce is derived). Several species were described in 50 AD and lettuce appeared frequently in medieval writings, including many herbs (Zohary *et al.*, 2012). Later, North America and Europe originally subdued the market for lettuce, but by the late 20th century era, lettuce consumption had spread around the world. In the year 2017, the world production of lettuce and chicory was twenty-seven (27) million tonnes, 56 percent of which produced from China (FAOSTAT, 2018). However, some species of lettuce were introduced in Australia and many countries in South America (POWO, 2019). The most prominent or well-known species of the genus is *L. sativa* L., with an annual production of 26.87 million tons in 2017 (FAO, 2017).

### 2.2 Botany of Lettuce

*Lactuca sativa* is an annual smooth herb with a thin taproot and an upright stem with a height of 30–100 cm that branches at the top (Doležalová *et al.*, 2012). *Lactuca sativa* leaves are spirally arranged, creating a dense rosette or a head before bolting. But their shape is rectangular to transverse elliptic, orbicular to triangular, undivided to pinnatisect (Doležalová *et al.*, 2012). The leaf margin is entire to setal dentate, often curly. Stem leaves are rectangular elliptic, with a cordate base. The inflorescence (capitulum, head) consists of 7–15 (35) yellow ligules (florets). The heads form a corymbose, densely leaflet panicle. The bract is 10–15 millimeters long, cylindrical; involucre bracts are generally to slim

lanceolate, light green, color with white margins, erect at the stage of fruit maturity (Doležalová *et al.*, 2012).

### **2.3 Varieties of lettuce**

Modern lettuce varieties can be divided into six main categories based on their morphological similarity, with the most common being Crisphead, Butterhead, Romaine, Cos, and Latin (Wei *et al.*, 2021). Crisphead lettuce (*var. capitata L. nidus jäggeri Helm*) is traditionally cultivated in soil and sold as a whole head salad, which is characterized by a dense, rosette-like head with leaves that form a single stalk. However, there is an increasing demand for hydroponically produced baby leaf lettuce, which is a type of lettuce that is ready to eat (Pinotti *et al.*, 2020). One of the most common types of lettuce is the crisphead lettuce, which is cultivated in many European countries, as well as in the United States and Brazil according to (Kim *et al.*, 2016 Noumedem *et al.*, 2017; Schwambach *et al.*, 2020).

The most common type of lettuce is the which belongs to the *Valerianaceae* (Caprifoliaceae) or valerian family, whose plants frequently have a strong and precise fragrance related to the presence of terpenes and organic acids. Isovaleric Acid is a volatile organic acid composed of five carbon atoms in a branched chain, which is primarily responsible for the distinctive aroma of Valerian (Lanje *et al.*, 2020b). lettuce (*Valerianella locusta L.* and *Valerianella eriocarpa Desv.*) is a type of plant that grows small each year and has a short growth cycle. It's popular for its sweet flavor and soft texture (García- Valcárcel *et al.*, 2016; Hernández *et al.*, 2021). It's made up of six or seven pairs of loose, opposing leaves that are usually harvested and eaten as whole rosettes (Ceglie *et al.*, 2018).

## **2.4 Production trend of Lettuce**

Lettuce is the second greatest appreciated salad produced in the United States of America with farm-market revenues of over \$1.98 billion within 2005 (USDA, 2005). Roughly 75% of all lettuce manufactured within the United State of America is cultivated within California, and combined with Arizona these two states account for 96% of the total lettuce production (USDA, 2005). China harvests nearly one-half of the world's lettuce and at over 11 million Metric tons, their production is in excess of double that of the United State (Table 2.1). After 1980 worldwide lettuce production has moved up to 2.7 fold with 22.4 million Metric tons produced within 2005 (FAO, 2006).

The increased production was primarily because of an increase in acreage planted as divergent to an increase in yield. Since 1980 to 2005, lettuce harvest (Kg/ha) increased by 118% compared to an increase of 149%, 135%, 146% and 128% for maize, potato, paddy rice and tomato, respectively (FAO, 2006). This could reveal the greater genetic resources established for these crops or alternatively, the greater yield gain of these other species might be an effect of selecting for the harvested organ (seed, fruit or tuber) although the vegetation growth in lettuce unable to additionally increased. In relations to biomass harvested per hectare, of all major agronomic and vegetable crops, only the tomato has a greater yield than lettuce (FAO, 2006). If vegetative growth has been maximized in lettuce, any increase in yield can only be derived from improving disease and insect resistance.

**Table 2.1: The top four lettuce producing countries and the world lettuce production for 2022**

countries	Millions of tonnes
China	15.0
United states	3.3
India	1.2
Spain	1.0
World	27.1

FAOSTAT. (2024)

## 2.5 Nutritional values of Lettuce

Although lettuce consumption is increasing, they are regarded as low in nutritional value. However, nutritional value of lettuce greatly differs with lettuce types depending on lettuce type, and nutrient composition can be comparable to other nutritious vegetables (Kim *et al.*, 2016). Lettuce contains many nutritive minerals important for human health such as phosphorus (P), magnesium (Mg), calcium (Ca), iron (Fe), zinc (Zn), manganese (Mn), and potassium (K) and other health-promoting bioactive compounds (Kim *et al.*, 2016). Lettuce is one of the most economically significant salad crop species, and its consumption has exploded in recent years due to its high nutritional value and phytochemical properties (Noumedem *et al.*, 2017 ; Pinotti *et al.*, 2020 ; Yang *et al.*, 2018). Lettuce is typically consumed raw as main course or as a side dish. As a result, it can significantly increase the nutritional value of a diet, as it retains more nutrients than other cooked/processed vegetables (Kim *et al.*, 2016). Consumption of salads, especially green leafy vegetable, have been associated with lower risk of a variety of diseases (Metabolic syndrome, type 2 diabetes, cardiovascular diseases, etc.,) (Hoy *et al.* 2019). The agronomic characteristics and nutritional qualities of lettuce or corn salad largely depend on the type, variety and cultivation technique of the salad, lettuce has a very brief postharvest shelf life and can be stored for as little as 15 days at temperatures close to 0°C, and up to 95% relative humidity (Kotsiras *et al.*, 2016). The nutritional quality of lettuce is affected by a variety of

environmental factors, such as light and temperature, growing season, agricultural practices, fertilizers, and storage conditions (Mou, 2012).

Lettuce are ideal foods from a nutritional point of view, as they are low-calorie, low-fat, and low-sodium. They are also good sources of essential nutrients (vitamins, minerals, etc.), as well as bioactive compounds (phenolic acid, flavonoid, glycosides, etc.) that promote health (Camejo *et al.*, 2020 ; Kim *et al.*, 2016). Phenolic compounds, like phenolic acid and flavonoids, are also found in leafy veggies and are thought to have antioxidant properties (Negrao *et al.*, 2021; (W. . Zhou *et al.*, 2020). The corn salad is part of the family Valerianaceae or Valerianaceae, and the plants often smell strong and have a specific odor associated with the presence of organic acids and terpenes. Valerian acid is a volatile organic acid with 5 carbon atoms, and it's mainly responsible for the aroma of valerian (Lanje *et al.*, 2020a). Valerian roots have valeric acids, but not isovaleric acid, according to (Shi *et al.*, 2021).

## **2.6 Bio stimulant**

A biostimulant is a non-fertilizer, non-pesticide substance that stimulates nutritional processes independent from the crop's nutritional content with the main objective for abiotic resistance, increasing nutrient use efficiency, quality trait, or the availability of limited nutrients in soil or the rhizosphere (Campobenedetto *et al.*, 2021 ; Yakhin *et al.*, 2017). These materials may be substances or microorganisms that promote plant growth, improve resistance to adverse environmental conditions, or encourage the optimal utilization of plant resources (Campobenedetto *et al.*, 2021 ; Yakhin *et al.*, 2017). Biostimulant substances are derived from a variety of sources, such as microorganisms, plant- or animal-derived by-products, recycled from the food sector, and seaweed extracts.

In recent years, seaweed-derived from biostimulants have been commercially available as substances that enhance plant growth. In normal environmental conditions, seaweed extracts may lead to an increase in crop productivity (Rouphael & Colla, 2020 ; Yakhin *et al.*, 2017). Throughout the past years the earth have observed marvelous growth in the utilization of bio stimulants in farming (Calvo *et al.*, 2014).

The European Biostimulants Industry Council (EBIC, 2012b) defined plant biostimulants as encompass element or materials or micro-organisms which perform as soon as applied to plants or the rhizosphere stimulate natural procedures to benefit or improve nutrient uptake, nutrient effectiveness, tolerance to abiotic stress, and crop quality. In North America, the Biostimulant Federation also defined biostimulants as “ingredients, including microorganisms, that are applied to seed, plant, soil or other growing media that could improve the plant’s capability to integrate applied nutrients, or provide aids or benefits to plant growth. Biostimulants are not plant nutrients and hence could not make any nutrient privileges or guarantees” (Biostimulant Coalition, 2013). Biostimulants are derived from an extremely different set of biological and inorganic materials (Calvo *et al.*, 2014) together with bacterial fermentations of animal or plant feedstock, macro, and micro-alga, living bacterial cultures, protein hydrolysate, fulvic, and humic substances, composts, manures, food, and industrial wastes prepared using widely divergent industrial manufacturing processes.

Biostimulants have been inspired for their capacity to stabilize abiotic stresses in plants and their mode of action is progressively studied. These products are capable of stabilizing environmental stress such as salinization, water deficit, soil, and exposure to suboptimal growth temperatures “(Van Oosten *et al.*, 2017 ; Rouphael, *et al.*, 2017a; Pokluda *et al.*,

2016 ; du Jardin, 2015; Desoky *et al.*, 2018; Di Stasio *et al.*, 2018; Masondo *et al.*, 2018; Ugena *et al.*, 2018)”. Salinity, specifically, is taken into consideration one of the key environmental factor that affects plant growth and metabolism in many Mediterranean regions, leading to excessive damage, turgor loss and excessive inhibition of growth (Borgognone *et al.*, 2014; Lucini *et al.*, 2015; Taïbi *et al.*, 2016; Roupael *et al.*, 2017).

## **2.7 Forms of Bio-stimulants**

### **2.7.1 Seaweed Extract Concentration**

Seaweeds, also known as macroalgae, are multi-celled marine algae that play an important role in marine coastal eco-systems. Seaweeds are classified into three main categories based on pigmentation: brown seaweeds (Phaeophyta), red seaweeds (Rhodophyta) and green seaweeds (Chlorophyta). Seaweed extracts contain excess of bioactive compounds. Some of the most common compound of seaweed extracts are: Pigments, Phenolic Compound, Polysaccharides, Proteins and (bioactive) peptides, phytohormones and Micro- and Macronutrients (Craigie, 2011 ; Stirk & Van Staden, 2014; El-Boukhari *et al.*, 2020) . Seaweed is a complex combination of protein, sugar, hormones, amino acid, lipid, vitamin, humic, and phenolic. The organic properties of seaweed and its physiological effects have resulted in seaweed being widely used in both pharmaceutical industries and food (Battacharyya *et al.*, 2015). Seaweed contains organic compounds, carbohydrates, and high levels of nitrogen, phosphorous, potassium and other minerals that enhance soil characteristics and are easily digested by plants. These extracts enhance plant growth and the antioxidant pool by stimulating the respiratory cycle and photosynthesis, thereby delaying plant ageing stated by Türkmen & Su (2019) . Di Mola *et al.* (2019) report that seaweed increases the growth and yield of lettuce and significantly improves fresh and dry

weight, stomatal conductance, potassium content and total antioxidant activity (Chrysargyris *et al.*, 2018).

Jung & Kim, (2020) stated increases in plant height and chlorophyll, carotenoids, total antioxidant activity and total antioxidant activity of lettuce using seaweed. The process in which the seaweed extracts are administered has a considerable influence on their utilization and plant reactions. Majority of application is either root application, foliar, or a combination of the two methods. Seaweed can be applied to soil or growing medium through drenching or drenched and fertigation (Jayaraman & Ali, 2015). It has been suggested that foliar sprays containing less than or equal to 0.05% of the extract v/v have been reported to be the most effective for the crop and provide more effective control of higher yields and disease (Ali *et al.*, 2016). The effectiveness of foliar applications can be attributed to the direct contact with plant tissues, as foliar absorption occurs almost instantaneously. Furthermore, extracts are commonly adsorbed by soil particles, which may limit its immediate mobility (Ali *et al.*, 2016 ; Ali *et al.*, 2020) In addition, it was found that the best time to use these extracts is approximately every 10-14 days to stimulate the best plant responses (Arioli *et al.*, 2015). Seaweed extract is considered to be one of the most promising biological substances for bio stimulation, and is currently the fastest-growing sector of the bio stimulation market (Carmody *et al.*, 2020; Markets & Markets, 2019).

### ***2.7.2 Mode of action and Effects of Seaweed Extracts on Plant Growth***

With Petunia plants, the use of seaweed extract recorded the highest plant height, (Elansary, 2017). Phytochemicals in seaweed extract such as gibberellins, abscisic acid, ethylene and auxins, auxins and cytokinins were responsible for elongating the growth of

plant tissue and for apical dominance and cell division. The cytokinins are involved in activating the cell divisions that are responsible for the growth of plants (Thomas *et al.*, 2013). Application of a low concentration (20%) seaweed foliar sprays showed significantly higher plant heights in maize plants compared to control. Higher concentrations (100%) had inhibitory effects on plant height because of the high salt index in higher concentration seaweed liquid extract (Sutharsan *et al.*, 2014). According to (Sutharsan *et al.*, 2014), maize seedling performance increases up to 20%, but decreases at higher concentrations. Spraying seaweed extract once a week increased plant height in two varieties of Pb-1 soybeans and MISB-01 soybeans (Bandara *et al.*, 2018). When seaweed extracts are introduced in different forms, they show a wide range of positive feedbacks such as increased germination, root growth, improved chlorophyll content, leaf area content, and resistance to the pathogens (Castellanos *et al.*, 2017).

Seaweed extract has been shown to increase the aggregation of plant biomass and yield when used as foliar sprays (Sutharsan *et al.*, 2014). Furthermore, (Spagnuolo *et al.*, 2021) found that *Kalanchoe daigremontiana*, *Carpobrotus edulis*, and *Kalanchoe tubiflora*, have the potential to significantly improve plant quality and increase vegetative and root biomass when treated with selected algae extracts. For macroalgae treatments, overall valuation including foliage, flowers as well as stem were enhanced (Leoni *et al.*, 2019). Seaweed extracts enhanced biomass, fresh shoot and dry weight of ornate sunflowers (Luan *et al.*, 2019). Marigold seedlings enhanced by concentrates of seaweed extracts seem to be most vigorous in texture as well as healthy (Karthikeyan & Shanmugam, 2015).

Prior to salinity stress exposure, Chickpea plants exposed to seaweed extracts showed significantly more vegetative growth than control Chickpea plants and are salt-stressed

plants without seaweed extracts (Abdel Latef *et al.*, 2017). Seaweed extracts were also found to enhance the growth of rice and wheat grown in saltwater environments (Zou *et al.*, 2018) ; Liu *et al.*, 2019). When cherry tomato seeds were pretreated with an *Ascophyllum nodosum*-based biostimulant, the quality of the fruit (measured as lycopene, flavonoids and total soluble solids) was improved significantly and the crop fruit yield increased by up to 65%, even in drought conditions (Murtic *et al.*, 2018). Similar results can be seen in two other studies of wheat grown in drought conditions. In these studies, foliar treatment using extracts from either *Gracilaria dura* (*Gracilaria* spp.) or *Kappafycus* spp., crop seed yield increased by 70% and 200% (twice the early yield), respectively (Sharma *et al.*, 2019) ; Trivedi *et al.*, 2018). Improvements were also observed in rapeseed plants that were treated with either of the following extracts: *Ulva Lactuca* extract; the results of a study conducted by Hashem *et al.* (2019) indicated that *Cystoseira* spp. *gelidium crinale* extract cultivated under either moderate (75 mM sodium chloride) or high (150 mM sodium chloride) salinity resulted an improvement. In this instance, the crop seed yield increased by 50% and 65% respectively under mild and severe salt stress, and the seed oil percentage also increased significantly compared to untreated plants (Hashem *et al.*, 2019). The Tomato plants that have been treated with two commercial extracts derived from the *Ascophyllum* showed better fruit quality under salt stress, which is defined as a substantial increase in vitamin C content (ascorbate) (Di Stasio *et al.*, 2018).

### **2.7.3 Humic Acids Concentration**

Research by Billingham, (2012); Hayes & Swift, (2020) stated that Humic substances are remains of decomposed plant and animal materials such as cellulose, tannins, lignin, and cutins. Humic substances are found in high amounts in soils after combining harvested residues (Wiesler *et al.*, 2016). The increased production of animals and biogas has

decreased the amounts of harvested residues on the majority of arable land.(Rose *et al.*, (2014); Gerke, (2018) stated that in recent decades, scholars have tried to replace the decreased humic substances with external application. Most of the external application of humic substances are commercially produced from coal, soil, and lignite, as well as from organic materials by (Gollenbeek & Van Der Weide, 2020 ; Yang *et al.*, 2021). De Melo *et al.* (2016) stated that, Humic substances are classified as humic acids, fulvic acids, and humin based on solubility in water, acidic, or alkaline solutions. Because the humin fraction of humic substances does not degrade, researchers have concentrated on the humic acid and fulvic acid fractions as they are able to improve soil fertility and health in a short time frame. The humic acid and the fulvic acid elements are chemically reactive and resist microbial reactions, thus performing beneficial roles in soils and plants (Billingham, 2012). About 60% of the humic acid fraction consists of C (organic carbon), which plays a significant role in soil microbial growth (Sible *et al.*, 2021). Humic acids, in addition to Carbon, are composed of nitrogen (N), oxygen (O), hydrogen (H), and sulfur (S) and play a variety of important roles in soil physical and biochemical processes.

They can improve the structure, texture, and water holding capacity of the soil, as well as the microbial population (Nardi *et al.*, 2017 ; Nardi *et al.*, 2021 ; Fuentes *et al.*, 2018 ; Shah *et al.*, 2018). Additionally, they can increase the availability of soil nutrients, particularly micronutrients, through chelating and co-transporting micronutrients to plant (Yang *et al.*, 2021). Furthermore, they can precipitate toxic heavy metals, thus reducing the amount of toxic heavy metals consumed by plant (Wu *et al.*, 2017). Again, Humic acids can also promote crop growth by increasing the production of plant growth promoting hormones, such as auxin, cytokinin, and other hormones, which are involved in photosynthesis, nutrient metabolism, stress resistance and other plant growth-promoting

processes (Billingham, 2012; Rose *et al.*, 2014 ; Canellas *et al.*, 2020 ; Laskosky *et al.*, 2020; Nardi *et al.*, 2021 ; van Tol de Castro *et al.*, 2021).

#### ***2.7.4 Effect of Humic Acids on soil and plant growth***

Continuous cultivation and planting of the same land year after year has a negative effect on soil structure and texture. Applying Humic acid has positive effects on the structure and texture of degraded soils according to Billingham, (2012) ; Yang *et al.* (2021). Increased soil structural stability is qualified to increased adhesion of humic acid to clay surfaces (Chen *et al.*, 2017). Adding humic acid results in chelates and cationic metals (Billingham, 2012). Cationic metals act as a link between the humic acid and the clay surfaces thus forming complexes. For instance, when bentonite-humic acid is applied to degraded sandy soil after 7 years continuous cropping with corn (Zhou *et al.*, 2019), it increases the macro-aggregate of the degraded soil. Coal-derived humic acid application does not enhance soil aggregate stability after two (2) growing cycles of maize research by Mukherjee *et al.* (2014). In both of the above experiments, the level of humic acid used was not sufficient to alter soil texture and structure as hypothesized by the authors. The soil used in the above studies was neutral to alkaline soil, which could potentially affect the bridging of humic molecules in the humic acid as confirmed in the review by Gerke, (2018). Furthermore, the humic acid source influences soil structure and soil texture (Rose *et al.*, 2014). It will therefore be important for researchers to evaluate the efficacy of a single source of humic acid without extrapolation to other sources. Additionally, humic acids have been demonstrated to enhance soil water holding capacity (Billingham, 2012; Yang *et al.*, 2021). The hydrophilic nature of humic acid, combined with improved soil structure, increases the water holding capacity of the soil. Furthermore, the combination of humic acid with fulvic acid has been reported to lead to the formation of colloids (humic

clay complexes) and increased water holding capacity (Billingham, 2012). In a study of 7-year field experiment to evaluate the effect of Bentonite Humic Acid on the water holding capacity of the soil, application of 30mg ha<sup>-1</sup> significantly increased the soil's water holding capacity. The increase in water holding capacity was more significant after the 4th year (Zhou *et al.*, 2019). Humic acid also increases the solute compatibility of plants (example: proline, glycine, and betaine) which is a strategy of adaptation for plants under water stress (El-Bassiouny *et al.*, 2014).

### ***2.7.5 Mode of action of Humic Acid on Plant Growth***

Studies have been conducted to assess the effects of Humic acid on plant growth and on agronomic parameters like root and shoot growth and leaf chlorophyll and yield. Humic acid stimulates root and shoot growth through increased production of plant growth promoting hormones like Auxin, Cytokinin, and metabolic enzymes (Rose *et al.*, 2014; Olaetxea *et al.*, 2020). The improved uptake of macro & micronutrients after Humic acid application increases leaf chlorophyll concentrations, which positively aids shoot growth (Fan *et al.*, 2014; Sible *et al.*, 2021). The production of enzymes and plant hormones and also an increase in chlorophyll content and photosynthesis rate, root & shoot weight after applying humic acid have been demonstrated to improve yields (Bybordi & Ebrahimian, 2013). According to Maji *et al.* (2017) the application of vermicompost-rich Humic acid was evaluated to significantly increase the length, height and weight of roots & shoots of pea when compared with NPK treatment alone. Application of Humic Acid derived from organic waste resulted in an increase in agronomic parameters of chrysanthemum such as leaf area, root & shoot dry weight, chlorophyll content, and yield (Fan *et al.*, 2014). Significant effect of different Humic acid rates and suggested NPK mineral fertilizer on

chlorophyll content, shoot and root weight, thousand grain weight, and grain yield in wheat reported by Arjumend *et al.* (2015).

In a field experiment, where NPK and different Humic acid rates significantly increased spike length, wheat plant height, thousand grain weight, and total yield; a major improvement was found at higher Humic acid application rates and moderate NPK rates (Khan *et al.*, 2010). El-Bassiouny *et al.* (2014) and (Mohammed *et al.*, 2019) conducted field experiments that Humic acid applied with NPK fertilizer significantly increased the chlorophyll content and shoot dry weight of wheat and *Stevia rebaudiana*; there was no significant difference in spike length and yield and wheat plant height in this experiment. Nasiroleslami *et al.* (2021) conducted a two-year field experiment in Iran to assess the effect of foliar application of different urea levels and Humic acid, where Humic acid treatment significantly improved the spike number, biomass, and yield of wheat at a urea application rate of 150Kg ha<sup>-1</sup> compared to 225Kg ha<sup>-1</sup>. In another two-year experiment with canola in Iran, the interaction of Humic acid-rich zeolite rates and different urea did not have an effect on the agronomic performance of canola, but urea and zeolite alone significantly improved agronomic parameters (Bybordi & Ebrahimian, 2013). The erratic outcomes suggested that the abovementioned studies show that recommendation for the use of Humic acid in improving crop agronomic performances can only be dependable after being tried under specific conditions. According to Rose *et al.* (2014) some literature suggests that combining the mineral fertilizers and Humic acid application form complexes that gradually release nutrients and ensuing crop uptake but the interaction effect depends typically on the Humic acid source, crop type and application rate.

Even though, high Humic acid dosages are related with improved soil physical characteristics (Gollenbeek & Van Der Weide, 2020), their effects on soil chemical characteristics and crops are still ambiguous (Rose *et al.*, 2014). A review by Rose *et al.* (2014), among the factors investigated in typically greenhouse trials, Humic acid source had substantial effects on both shoot growth and root while the application rate only meaningfully affected shoot growth. The predominant features of Humic acid in the soil are the COOH (Carboxylic Acid) and the phenolic (H<sub>2</sub>O<sub>2</sub>) group. A review by De Melo *et al.* (2016) stated that the COOH and phenolic groups play a major role in the function of Humic acids in soil. According to a recent review of Nardi *et al.* (2021), molecular structure and the chemical of Humic acids, their sources, and their application rates are important factors in determining their impact on soils and crops. It is important to note that application of Humic acid may produce inconsistent results in terms of yield, which may be attributed to the different biological origins of Humic acids (Sible *et al.*, 2021).

#### ***2.7.6 Fulvic Acids Concentration***

As debated above in the section on humic acids, humic substances are divided into different types that involves humic acids, fulvic acids, and humins (Berbara & García, 2014). The choice to separate humic acid and fulvic acid into two types for this analysis was made since many of the experimental and commercial bio stimulants now being advanced or marketed are acknowledged specially as either humic acids or fulvic acids. On the contrary, the scientific literature comprises information that stipulate humic or fulvic acid in addition to information that state generally to humic substances (Zhou *et al.*, 2019; Liu *et al.*, 2022).

Fulvic Acid (FA) is a commonly used amendment in soils environments which has been shown to be beneficial to promote plant growth and increase crop yields (Li *et al.*, 2021 ; Chen *et al.*, 2021). Depending on the extraction source, fulvic acid can be classified as natural and biochemical. Natural fulvic acid is an organic, macromolecule, weak acid mixture formed through a series of decompositions and trans-formations of animal and plant residues that undergo microbial, geophysical, and chemical action which can be extracted from soils, water and coal (Canellas *et al.*, 2015). Biochemical fulvic acid a type of weak acid organic matter that can be extracted from agricultural waste by using microbial fermentation technology or by straw-based ammonium sulfite pulping process (Yao *et al.*, 2019). It has similar chemical properties to natural fulvic acid (Liu *et al.*, 2022; Chen *et al.*, 2021). This is attributed to several functional groups of the biochemical fulvic substance these functional groups include carboxylic Acid. Earlier studies have shown that fulvic acid results from the breakdown of the plant lignin into compounds (Sabahi *et al.*, 2017). However, because of the apparent involvement of several impurities, low availability, and easy transformation in soil, the majority of plant polyphenols are limited to the experimental phase and are not considered to be suitable for promotion in crop production. Fulvic acid isolated from some plant polyphenols contains a combination of soluble organic acids produced by polymerization of chemically as well as biologically active functional groups, influences N cycling and has high potential to promote crop growth and influence succedent validity for urea.

Phenolic hydroxyl group and quinone group of Fulvic acid have been shown in some research to undergo redox reactions with urea sulfhydryl to form fulvic acid-urea complexes, Thus, complexes inhibit urea activity and delaying the conversion and degradation of urea. (Liu *et al.*, 2016 ; Liu *et al.*, 2019). However, Functional Groups

(Carboxyl and Phenolic Hydroxyl) in fulvic acid compete with Phosphorus (P) in the adsorptive sites on mineral surfaces, resulting in a decrease in Phosphorus fixation and an increase in Phosphorus availability (Yang *et al.*, 2021). Fulvic acid application increases maize lateral root mitotic sites and increases H<sup>+</sup>-ATPase activity in the root plasma membrane. This stimulates root growth and improves the nutrient uptake capacity of maize (Zandonadi *et al.*, 2019). In addition to the above, fulvic acid up-regulates maize root nitrate uptake & assimilation genes, promotes nitrate absorption, and improves N assimilation efficacy (Vaccaro *et al.*, 2015).

## **2.8 Effect of Fulvic Acid on Plant growth**

Fulvic acid (FAs) enhance soil structure and fertility in soils with a variety of textures and play an essential role in augmenting crop yield (Kumar *et al.*, 2020). Humic acid in combination with fulvic acids converts minerals to organic compounds that are easily digested by plants (Kumar & Alope, 2020). Additionally, the application of Fulvic Acid (FA) to foliage increases the iron absorption and growth of (*Lactuca Sativa* L.) plants under cadmium pressure (Wang *et al.*, 2019). Fulvic acids have been found to be beneficial in the growth and yield of onion plants (*Allium* *capa* L.), among others, which has been soaked in fulvic acids to improve vegetative development (Elansary *et al.*, 2020). Additionally, fulvic acids have been demonstrated to be effective in the production of crops produced in polluted industrial areas, including wheat, according to Ali *et al.* (2018). However, in the case of *Lepidium sativum* plants, the presence of humic acid and fulvic acid in high concentrations has been found to reduce the uptake of chlorines and cadmias (Yildirim *et al.*, 2021).

Furthermore, fulvic acid has been found to contain a high proportion of oxygen-containing functional groups (e.g., hydroxyl, phenolic, carbonyl, and carboxyl), which can be used to facilitate the complexation of metal oxides and heavy metals (Caradonia *et al.*, 2019).  $\text{Cd}^{2+}$  is an abundant organic material in the environment and can increase its mobility and bioavailability through competitive complexation, resulting in Fulvic acid heavy metal ion (FA-HMI) complexes with high solubility, as demonstrated by Ertani *et al.*, (2015). Cadmium toxicity has been identified in grown lettuce (Lau *et al.*, 2022). Therefore, it is hypothesized that exogenous Fulvic acid could decrease the speciation, and bioavailability of Cu and Cd in the topsoil. Fulvic acid has been shown to enhance nutrient uptake in crops according to de Saeger *et al.* (2020). Spraying fulvic acid has been shown to promote crop growth and improve fruit quality (Nephali *et al.*, 2020). Fulvic acid has been shown to have the following significant increase in: Chlorophyll content Leaf photosynthetic rate, Nitrogen use efficiency, stimulation of plant growth and increase the yield of maize plant (Council of the European Union, 2018). Fulvic acid could enhance seed germination and root, stem, leaf development spring wheat and other crops and improve the quality and yield of agronomic produce (Rouphael & Colla, 2020). According to Zulfiqar *et al.* (2020) fulvic acid could improve vegetative characteristics, improve yield, nutrient uptake capacity of rice.

Fulvic Acid (FA) has been demonstrated to promote plant growth due to its low molecular weight (Canellas & Olivares, 2014), high functional group content, and ease of transmissibility through cell membranes (Qin *et al.*, 2016b). Additionally, fulvic acid has been demonstrated to alter the primary and secondary metabolic pathway of plants related to abiotic stress tolerance. Fulvic-acid-like component of black liquor (PFA) was similar to Fulvic acid extracted from Leonardite (a non-renewable source). They have similar

elemental compositions and functional groups as stated by Wang *et al.* (2019). Fulvic Acid (FA), which is the most abundant organic material in soil, is studied in agriculture, soil chemistry, soil fertility and plant physiology, as well as in environmental sciences, due to the multiple roles it plays in the growth of plants (Zhang *et al.*, 2021). Fulvic acid positive effects on plant growth can be due to its ability to increase fertilizer efficiency or improve plant biomass (Ahmad *et al.*, 2018). Fulvic acid also has the ability to reduce the stress of heavy metal on the growth of plant (Yildirim *et al.*, 2021) and to increase the yield by improving the nutrient status of the soil (Ali *et al.*, 2022).

Fulvic acid is a naturally occurring compound that improves soil physicochemical and bioavailability (Akhtar *et al.*, 2014; Tuba *et al.*, 2015). It also improves soil conditions and brings balance to the plant (Zhang *et al.*, 2021), plant residues are the primary source of plant nutrients, as well as the essential mechanisms for the stability of agricultural systems. According to Esringu *et al.* (2015) the increase in stem diameters and spike weights may be a result of the positive effects of Fulvic acid on plant growth parameters, and may also be due to nutrients provided by these changes. Fulvic acid may improve plant growth parameters, nutrient uptake, and reduce the perceived presence of harmful mechanisms and enhance plant metabolism (Sootahar *et al.*, 2019). Fulvic acid and Humic acid also improved yield and yield components of wheat and various grain-producing crops example (1000-grain weight; biological yield; dry matter; grain yield; and harvest index) were significantly affected by humic acid (Qin *et al.*, 2016; Tuba *et al.*, 2015).

In addition to the above, fulvic acid aid acts as a soil conditioner, improving soil quality and promotes plant growth (Kapooore *et al.*, 2021; Xu *et al.*, 2019). The result of aerobic decomposition is a range of organic matter predominantly composed of biochemically

humic acid and biochemically fulvic acid, with the latter having a higher content of carboxyl group and phenolic group compared to the former (Jindo *et al.*, 2016 ; Liu *et al.*, 2019). According to Li *et al.* (2021), fulvic Acid (FA), is a type of water-soluble polymer with the lowest molecular weight, highest physiological activity, and a wide range of functional groups within Humic acid. Fulvic acid are therefore aids in the uptake of nitrogen by plants, inhibits soil fixation of phosphorous and potassium, induces soil aggregates formation, and improves soil physical and chemical properties. Fulvic acid also improve photosynthesis, promotes crop growth, regulates stomatal openness, decreases transpiration, and enhances drought resistance. Fulvic acid is a class of humic acid with a more prominent peripheral part and less prominent aromatic core. Fulvic acid dissolves well in alkaline solution, acids and water. Humification starts with the formation of fulvic acids (Izosimov, 2016). Fulvic acid also influences enzyme activity in grain germination. Fulvic acid (FA)-Molecular Weight below 500 (Mw) as a chemical composition has been analyzed and its activity in promoting wheat seed germination has been studied (Qin *et al.*, 2016a). FA-500 was obtained by membrane separation technology and quantitatively and qualitatively analyzed by gas chromatography-mass spectrometry coupled with retention index. However, the best concentration of 0.5% and showed an inhibitory effect with increasing concentration.

According to Qin *et al.* (2016a), through seed germination, fulvic acid-500 can influence seed growth by affecting amylase activity, which is related to respiration. Canellas *et al.* (2015) stated that humic acid (HA) and fulvic acid (FA) are chemically complex humic compounds that are obtained from the chemical and biochemically transformation of plant matter and animal matter. Since these compounds are also naturally occurring in nature, the potential benefits of these compounds on plant physiology are well known. However,

their use in horticulture is mainly tested in the last decade (Canellas *et al.*, 2015). To produce commercial formulations containing humic acid (HA) and/or fulvic acid (FA), these compounds are obtained from various sources such as compost, peat, lignite, soil and raw organic waste (Rose *et al.*, 2014).

## **CHAPTER THREE: MATERIALS AND METHODS**

### **3.1 Experimental Location**

This study was conducted at the multipurpose crop nursery of the Akenten Appiah-Menka University, Mampong Campus in Asante Mampong Municipality of Ashanti Region, Ghana between 10<sup>th</sup> December 2021 to 14<sup>th</sup> January 2022. Ashanti Mampong lies at the Forest-Savannah Transitional zone between the forest and northern savanna zone of Ghana. Ashanti Mampong lies at 57.6km in Kumasi on the latitude 0.7°, 0.4° N of the equator and longitude 1°, 0.24° W of the equator. It is also situated at an elevation of 457.5m above sea level (Geodatos., 2023).

### **3.2 Climate**

The Mampong area experiences an annual rainfall ranging between 1094 mm and 1200 mm, with mean daily and monthly temperatures of 30.5<sup>0</sup>C and 32.0<sup>0</sup>C, respectively. The major rains begin around early April and end in July whilst the minor rains start in September and end in November. There is a short dryness in August but the main dry season begins in December and ends in March (Ghana Statistical Service, 2014).

### **3.3 Soil Type**

The soil type in the study area falls under Bediese series, which is sandy loam, well-drained, and has a thin layer of organic matter. The soil is classified as Ochrosol formed from Voltaian sandstone, and its pH ranges from 6.0 to 6.5 (Essilfie, 2015).

### **3.4 Vegetation**

Asante Mampong has a semi-deciduous type of vegetation with a dense grass cover. The soil of the area is suitable for growing many vegetables such as carrots, pepper, lettuce,

sweet pepper and commercial crops such as yam, cassava, cocoa, cashew, maize and plantain also do well on the soil. The soil can be tilled either by manual or by mechanical preparation (Ministry of Food and Agriculture, 2015).

### **3.5 Planting Materials**

Seeds of three varieties of lettuce (*Lactuca sativa*) were used. The varieties were Eden, Omega and Great Lakes. The seeds were obtained from K. Badu Agrochemicals in Kumasi of the Ashanti region.

### **3.6 Land Preparation**

An area measuring 27 m x 5 m was measured, demarcated and ploughed. Thereafter, the designed layout for the experiment was lined and pegged on the 24<sup>th</sup> November, 2021.

### **3.7 Research design, treatment and field layout**

#### ***3.7.1 Research design***

A 3x3 factorial was laid out in Randomized Complete Block Design (RCBD) with three replications.

#### ***3.7.2 Factors***

##### **A (Bio stimulant)**

1. Seaweed humic concentration
2. Fulvic humic acid concentration
3. No stimulant

##### **B (Variety)**

1. Eden (V1)

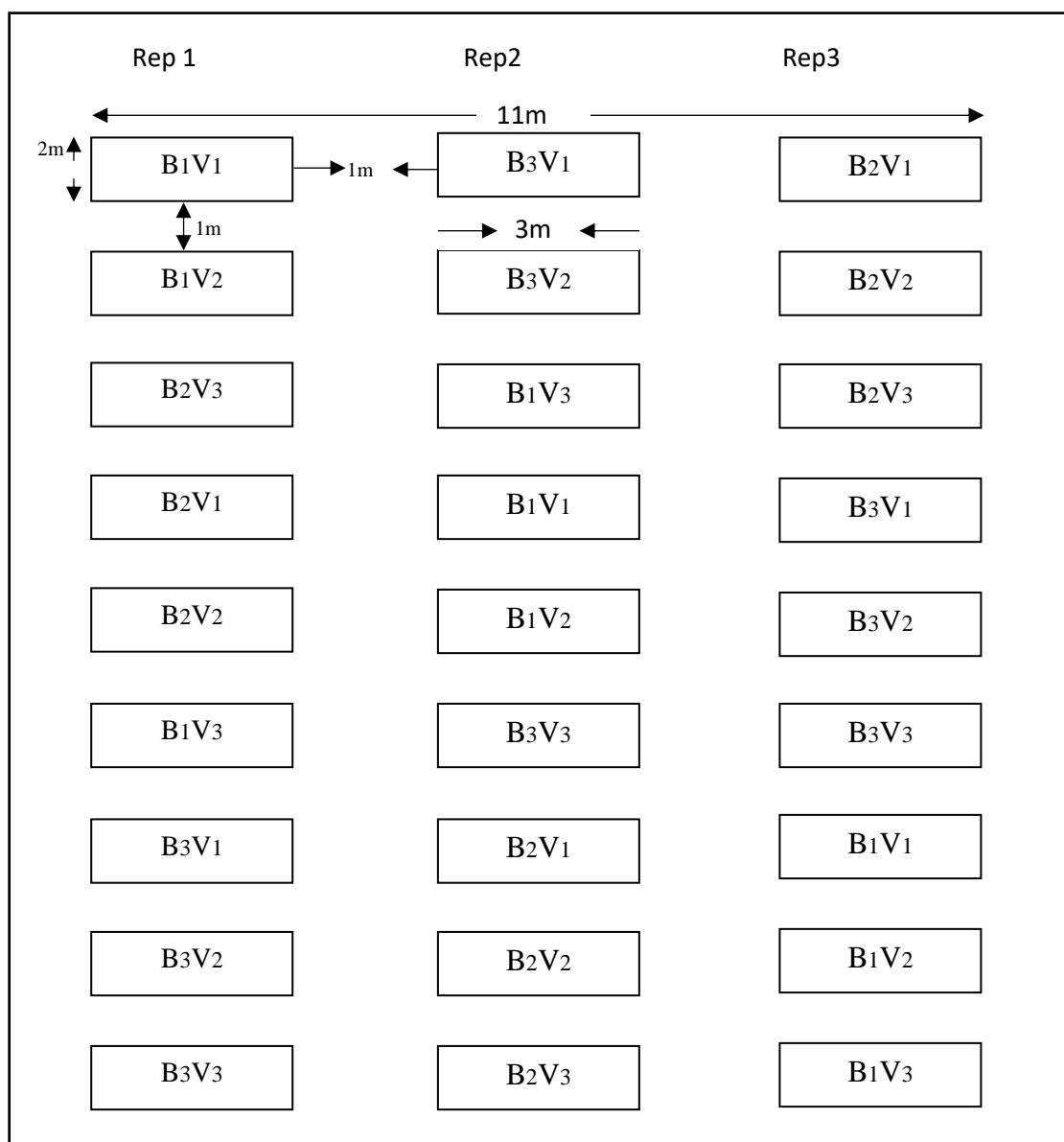
2. Omega (V2)
3. Great lakes (V3)

### ***3.7.3 Treatment combinations***

1. Seaweed Humic Concentration (B1) + Eden variety (V1)
2. Seaweed Humic Concentration (B1) + Omega variety (V2)
3. Seaweed Humic Concentration (B1) + Great Lakes variety (V3)
4. Fulvic Humic Acid Concentration (B2) + Eden variety (V1)
5. Fulvic Humic Acid Concentration (B2) + Omega variety (V2)
6. Fulvic Humic Acid Concentration (B2) + Great Lakes variety (V3)
7. No stimulant (B3) + Eden variety (V1)
8. No stimulant (B3) + Omega variety (V2)
9. No stimulant (B3) + Great Lakes variety (V3)

### ***3.7.4 Field layout***

The total experimental area was 15m × 5m. Each experimental plot measured 2m × 3m with 1m in between blocks and within plots.



**Figure 3.1 Field layout of treatment combination**

### **3.8 Cultural practices**

#### **3.8.1 Nursery**

The lettuce seeds were nursed on a well-prepared bed on 10<sup>th</sup> November, 2021. The seeds were drilled on a bed 5m long and thinly covered with soil which was later mulched with palm fronds and watered lightly. Germination occurred between fourth to fifth day after drilling. The mulching material were completely removed and a shed was erected on the

bed to protect the growing seedlings from the direct impacts of the sun. The seedlings were watered every morning and evening.

### ***3.8.2 Transplanting***

Transplanting was done four (4) weeks after nursery. Healthy plants were selected and transplanted to the main field while poor plants were left on the nursery bed. Transplanting was done in the evening of 10th December, 2021. Each plot was made up of five rows with five plants per row giving a planting density of 25 plants per plot.

### ***3.8.3 Application of Bio stimulant***

One week after transplanting, the application of biostimulants was done at a rate of 5ml/plant for each treatment requirement. The biostimulants were applied using the side placement method. A circle was drawn around the base of the plant and the biostimulants were carefully spread in the groove. The circle was made 2 inches reasonably far from the stem of the plant.

### ***3.8.4 Watering***

Watering was done twice daily morning and evening after transplanting up to maturity.

### ***3.8.5 Weed Control***

Weeds were controlled by weeding and hand picking. Four (4) separate weeding's were carried out at one (1) week intervals. This was to ensure that weeds did not compete with the crop for nutrients, water and sunlight.

### ***3.8.6 Disease and pest control***

Incidence of disease and pest were very minimal so no control measures were taken against it.

### ***3.8.7 Harvesting***

Harvesting was done six (6) weeks after transplanting. The lettuce plants were pulled out from the soil after being loosened with a knife and cutlass.

## **3.9 Data Collection**

Data were collected on the five (5) sampled plant from two central rows at one-week intervals. The data collected were number of leaves, leaf length, canopy spread, and dry matter accumulation.

### ***3.9.1 Number of leaves***

The number of leaves from the five (5) randomly selected and tagged plants in the two (2) central rows were counted and their mean values were calculated and recorded. This was done every one week's interval after transplanting.

### ***3.9.2 Leaf length (cm)***

Using the meter rule, five (5) random leaves from each tagged plant was selected and measured from the base to the apex of the uppermost leaf. The mean values of these measurements were then calculated and recorded.

### ***3.9.3 Canopy Spread***

The spread of the canopy of five (5) tagged plant randomly selected from the two middle rows per plot was measured using the meter rule from one end to the other of the widest canopy at one (1) week intervals and the mean was computed.

### ***3.9.4 Dry Matter Accumulation (g)***

One (1) plant was removed from the boarder rows of each plot at one-week interval after transplanting. The roots were separated from the shoots and weighed fresh on Westinghouse electronic balance. These were then dried in the oven for 48hrs at 75<sup>0</sup>C. Thereafter, dried shoots and root weight were measured and recorded.

### **3.10 Statistical Analysis**

All the data collected were entered into Microsoft Office Professional 2016 and later exported to GenStat statistical software for Analysis of variance (ANOVA). Treatment means were separated with LSD at a probability of 5%.

## CHAPTER FOUR: RESULT AND DISCUSSION

### 4.1 RESULTS

#### 4.1.1 Number of leaves

Table 4.1 shows no significant ( $p>0.05$ ) difference among (V1) Eden, (V2) Omega and (V3) Great Lake varieties of lettuce at first week after transplanting (WAT). However, in week 3 and 4 the number of leaves of Great Lake and Omega were statistically the same but significantly different from that of Eden. Upon reaching 5 WAT, Omega became statistically similar to Eden. However, Eden proved best in all the WAT. On the levels of biostimulant, no statistical differences were observed among seaweed with humic, fulvic humic acid and the control at 1WAT (3.21, 3.15 and 3.21) (Table 4.1). But seaweed with humic (B1) and no biostimulant (B3) recorded a consistent significant difference along the weeks when compared to fulvic humic acid (B2). However, seaweed with humic acid (B1) produced more leaves than no biostimulant (B3). Seaweed humic concentration with Eden variety (V1B1) recorded a significantly higher number of leaves on the 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> week after transplanting. But was statistically similar to all the treatment combination with the exception of no stimulant with Omega (V2B3) on the 3<sup>rd</sup> week after transplanting. However, the 4 WAT Seaweed humic concentration with Eden variety (V1B1) was Seaweed humic concentration with Eden variety (V<sub>1</sub>B<sub>1</sub>) only statistically similar to Seaweed humic concentration with omega variety (V<sub>2</sub>B<sub>1</sub>), Seaweed humic concentration with great lakes variety (V<sub>3</sub>B<sub>1</sub>), fulvic humic acid concentration with Eden variety (V<sub>1</sub>B<sub>2</sub>), no stimulant with Eden variety (V<sub>1</sub>B<sub>3</sub>), no stimulant with Omega variety (V<sub>2</sub>B<sub>3</sub>) and statistically similar to no stimulant with Eden variety (V<sub>1</sub>B<sub>3</sub>), no stimulant with Omega variety (V<sub>2</sub>B<sub>3</sub>) and seaweed humic concentration with Omega variety (V<sub>2</sub>B<sub>1</sub>) on the 5WAT. No stimulant with Great lakes variety (V<sub>3</sub>B<sub>3</sub>) produced the lowest number of

leaves in all the WAT. There were no significant difference among all the treatment combinations in the 2<sup>nd</sup> WAT (Table 4.1).

**Table 4.1 Results of variety and Bio stimulant on number of leaves of lettuce**

	Number of Leaves			
	2WAT	3WAT	4WAT	5WAT
<b>Variety</b>				
Eden (V1)	3.17a	6.17b	8.47b	11.29b
Omega (V2)	3.10a	6.02ab	8.20ab	11.22b
Great lakes (V3)	3.30a	5.54a	7.21a	9.21a
<b>LSD (0.05)</b>	0.31	0.60	1.01	1.39
P-vallue	<0.001	<0.001	<0.001	<0.001
<b>Bio stimulant</b>				
Seaweed with humic (B1)	3.21a	6.27b	8.77b	11.80b
Fulvic humic acid concentrate (B2)	3.15a	5.93ab	7.21ab	9.78a
No stimulant (B3)	3.21a	5.53a	7.89a	10.15a
<b>LSD (0.05)</b>	0.31	0.60	1.01	1.39
P-value	<0.001	<0.001	<0.001	<0.001
<b>Variety x Bio stimulant</b>				
V1×B1	3.20a	6.37b	9.13d	13.08d
V2×B1	3.10a	6.33b	8.33bcd	11.73cd
V3×B1	3.33a	6.10b	8.83cd	10.58abc
V1×B2	3.26a	6.07b	7.60abcd	10.13abc
V2×B2	2.93a	5.73b	7.33abc	10.33abc
V3×B2	3.26a	6.00b	6.70ab	8.87ab
V1×B3	3.06a	6.07b	8.67cd	10.67bcd
V2×B3	3.26a	6.00b	8.93cd	11.60cd
V3×B3	3.30a	4.53a	6.08a	8.18a
<b>LSD (0.05)</b>	0.54	1.04	1.75	2.41
<b>P-value</b>	<0.001	<0.001	<0.001	<0.001
<b>CV (%)</b>	9.8	10.2	12.7	13.2

*Means followed by or sharing the same letters within a column are not significantly different at 5% level of significance, CV=coefficient of variation, LSD=least significant difference at 5%, WAT = Week after transplanting.*

#### ***4.1.2 leaf length***

A significant ( $p < 0.05$ ) difference was seen between Eden and Omega and that of Great Lake along the weeks with the exception of week 5 where statistical difference existed only between Eden and Great Lake. Omega variety recorded the highest leaf length on the 2WAT, Eden 3WAT and Omega was on both 4 and 5WAT (Table 4.2). On the levels of biostimulant, no statistical differences were observed among seaweed with humic (B1), fulvic humic acid (B2) and the control, (B3) from the 2WAT to 4WAT. However, B1 recorded the longest leaf throughout the week.

Meanwhile on the 5WAT no statistical difference was observed between fulvic humic acid (B2) and no stimulant (B3) as well as seaweed humic concentration (B1) and fulvic humic acid concentration (B2). Overall, B1 improved the leaf length of lettuce throughout the WAT (Table 4.2). When the factors were combined, Seaweed humic concentration with Omega variety (V2B1) recorded a significantly higher leaf length at the end of the 5WAT after transplanting, but was statistically similar to Seaweed humic concentration with Eden variety (V1B1), Seaweed humic concentration with Great lakes variety (V3B1), fulvic humic acid concentration with Eden variety (V1B2), fulvic humic acid concentration with Omega variety (V2B2), fulvic humic acid concentration with Great lakes (V3B2), no stimulant with Eden variety (V1B3) and no stimulant with Omega variety (V2B3). Thus, no stimulant with Great Lakes variety (V3B3) recorded the lowest leaf length. It was statistically similar to seaweed humic concentration with Eden variety (V1B1), seaweed humic concentration with Great Lake variety (V3B1), fulvic humic acid concentration with Eden variety (V1B2), fulvic humic acid concentration with Great Lakes variety (V3B2), no stimulant with Eden variety (V1B3) and no stimulant with Omega variety (V2B3). While seaweed humic concentration with Omega variety (V2B1) recorded the highest leaf length

along the weeks. No stimulant with Great Lake variety (V<sub>3</sub>B<sub>3</sub>) recorded the lowest leaf length along weeks (Table 4.2).

**Table 4.2 Results of variety and Bio stimulant on Leaf length of lettuce**

	Leaf length (cm)			
	2WAT	3WAT	4WAT	5WAT
<b>Variety</b>				
Eden (V1)	3.19b	5.75b	7.40b	8.30b
Omega (V2)	3.33b	5.67b	8.02b	8.98ab
Great lakes (V3)	2.24a	4.09a	6.18a	7.80a
<b>LSD (0.05)</b>	0.75	1.04	1.21	1.11
<b>P-value</b>	<0.001	<0.001	<0.001	<0.001
<b>Bio stimulant</b>				
Seaweed with humic (B1)	3.04a	5.58a	7.93a	8.99b
Fulvic humic acid concentrate (B2)	2.90a	4.92a	6.89a	8.26ab
No stimulant (B3)	2.82a	5.01a	6.78a	7.83a
<b>LSD (0.05)</b>	0.75	1.04	1.21	1.11
<b>P-value</b>	<0.001	<0.001	<0.001	<0.001
<b>Variety x Bio stimulant</b>				
V1×B1	3.39b	6.02bc	7.95bc	8.80ab
V2×B1	3.46b	6.03bc	8.03c	9.62b
V3×B1	2.28ab	4.69abc	7.82bc	8.56ab
V1×B2	2.90ab	4.80abc	6.74abc	7.98ab
V2×B2	3.09b	5.45bc	8.04c	8.90b
V3×B2	2.71ab	4.51ab	5.90ab	7.89ab
V1×B3	3.28b	6.44c	7.51bc	8.13ab
V2×B3	3.46b	5.52bc	8.01c	8.43ab
V3×B3	1.73a	3.07a	4.83a	6.94a
<b>LSD (0.05)</b>	1.30	1.80	2.09	1.92
<b>P-value</b>	<0.001	<0.001	<0.001	<0.001
<b>CV (%)</b>	25.7	20.2	16.8	13.3

*Means followed by or sharing the same letters within a column are not significantly different at 5% level of significance, CV=coefficient of variation, LSD=least significant difference at 5%, WAT = Week after transplanting.*

### ***4.1.3 Canopy spread***

Table 4.3 shows no significant ( $p>0.05$ ) difference between Eden and Omega variety of lettuce from week 1-4. However, at week 5 the canopy spread of Great Lake and Eden were statistically the same but different from that of Omega. In all, Eden produced the highest canopy spread. On the levels of biostimulant, no statistical differences were observed among seaweed with humic (B1), fulvic humic acid (B2) and the control (B3) at 1WAT (3.39, 3.53 and 3.37). Meanwhile seaweed humic concentration (B1) and the control (B3) recorded a consistent significant along the weeks when compared to the control (B3). Generally, seaweed with humic acid better in all the weeks of study (Table 4.3). There was also interaction effect between the two factors under study (Table 4.3). seaweed humic concentration with Omega (V2B1) recorded a significantly higher canopy spread in 5<sup>th</sup> week after transplanting but was statistically similar to seaweed humic concentration with eden variety (V1B1), seaweed humic concentration with great lakes variety (V3B1), fulvic humic acid concentration with eden variety (V1B2), fulvic humic acid concentration with omega variety (V2B2), no stimulant with eden variety (V1B3), and no stimulant with omega variety (V2B3). Whiles seaweed humic concentration with eden variety (V<sub>1</sub>B<sub>1</sub>) recorded the highest canopy spread from the 2 -4 WAT no stimulant with great lakes variety (V<sub>3</sub>B<sub>3</sub>) recorded the lowest canopy spread in all WAT.

**Table 4.3 Results of variety and Bio stimulant on Canopy spread of lettuce**

VARIETY	canopy spread (cm)			
	2WAT	3WAT	4WAT	5WAT
EDEN (V1)	3.11b	11.28b	15.09b	16.26ab
OMEGA (V2)	2.67b	11.10a	15.66b	17.93b
GREAT LAKES (V3)	1.67a	8.04a	11.82a	13.92a
LSD (0.05)	0.30	1.32	2.62	2.34
P-value	<0.001	<0.001	<0.001	<0.001
Bio stimulant				
SEAWEED WITH HUMIC (B1)	2.76b	11.21b	16.43b	17.69b
FULVIC HUMIC ACID CONCENTRATION (B2)	2.07a	9.66a	12.99a	15.08a
No stimulant (B3)	2.62ab	9.56a	13.14a	15.35ab
LSD (0.05)	0.30	1.32	2.62	2.34
P-value	<0.001	<0.001	<0.001	<0.001
Variety x Bio stimulant				
V1×B1	3.53d	12.13d	16.81c	16.75cd
V2×B1	2.90cd	11.93d	16.58c	19.39d
V3×B1	1.87abc	9.57bc	15.91c	16.92cd
V1×B2	2.58bcd	10.10bcd	12.91bc	15.10abc
V2×B2	1.91abc	10.23bcd	14.85bc	17.30cd
V3×B2	1.72ab	8.63b	11.22ab	12.84ab
V1×B3	3.22d	11.63cd	15.55bc	16.92cd
V2×B3	3.22d	11.13cd	15.54bc	17.11cd
V3×B3	1.43a	5.93a	8.32a	12.00a
LSD (0.05)	0.52	2.28	4.54	4.06
P-value	<0.001	<0.001	<0.001	<0.001
fCV (%)	25.9	13.0	18.5	14.7

Mean followed by or sharing the same letters within a column are not significantly different at 5% level of significance CV= coefficient of variation, LSD= least significant difference at 5%, WAT = Week After Transplanting.

## 4.2 Dry matter accumulation

### 4.2.1 Dry Root Weight

Table 4.5 shows no significant ( $p>0.05$ ) difference between Eden and Omega variety of lettuce from the 3<sup>rd</sup> -5<sup>th</sup> WAT. Meanwhile on the 3<sup>rd</sup> WAT Eden, Omega and Great lakes were statistically similar but Great lakes was significantly different from that of Eden and Omega at 4<sup>th</sup> and 5<sup>th</sup> WAT. On the levels of biostimulant, statistical differences were not observed among seaweed with humic (B1), fulvic humic acid (B2) and the control (B3) at

3WAT and 5WAT. However, on week, seaweed humic concentration (B1) was significantly higher dried root weight than that of the control (B3). Meanwhile fulvic humic acid concentration (B2) and control (B3) were statistically the same as well as seaweed humic concentration (B1) and fulvic humic acid concentration (B2) (Table 4.5). A combination of the factors also showed significant difference in all the weeks after transplanting. No stimulant with omega variety (V2B3), seaweed humic concentration with eden variety (V1B1) and fulvic humic acid concentration with omega variety (V2B2) recorded a significantly higher dry root weight on the 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> week after transplanting. While, V3B3 recorded the least dry root weight on 3<sup>rd</sup> and 4<sup>th</sup> weeks after transplanting and fulvic humic acid concentration with great lakes variety (V3B2) on the 4<sup>th</sup> WAT (Table 4.5).

**Table 4.5 Bio stimulant and varietal effect on dry root weight of lettuce**

	Dry root weight (g)		
	3WAT	4WAT	5WAT
Variety			
Eden (V1)	0.15a	0.27a	0.41a
Omega (V2)	0.16a	0.26a	0.43a
Great lakes (V3)	0.13a	0.15b	0.32b
LSD (0.05)	0.04	0.06	0.08
P-value	<0.001	<0.001	<0.001
Bio stimulant			
Seaweed with humic (B1)	0.13a	0.25a	0.39a
Fulvic humic acid concentrate (B2)	0.16a	0.25ab	0.41a
No stimulant (B3)	0.16a	0.18b	0.34a
LSD (0.05)	0.04	0.06	0.08
P-value	<0.001	<0.001	<0.001
Variety x Bio stimulant			
V1×B1	0.14ab	0.35a	0.52a
V2×B1	0.13ab	0.26abc	0.34bc
V3×B1	0.11b	0.16cd	0.33bc
V1×B2	0.14ab	0.18bcd	0.34bc
V2×B2	0.18ab	0.35abc	0.53a
V3×B2	0.17ab	0.13d	0.39abc
V1×B3	0.16ab	0.29a	0.38abc
V2×B3	0.19a	0.28ab	0.41ab
V3×B3	0.12b	0.16cd	0.24c
LSD (0.05)	0.06	0.11	0.15
P-value	<0.001	<0.001	<0.001
CV (%)	39.98	28.49	22.88

*Means followed by or sharing the same letters within a column are not significantly different at 5% level of significance, CV=coefficient of variation, LSD=least significant difference at 5%, WAT = Week after transplanting*

#### ***4.2.2 Dry shoot weight (g)***

Table 4.6 shows a significant ( $p < 0.05$ ) difference between Eden and Omega variety of lettuce from the 3<sup>rd</sup> -4<sup>th</sup> WAT. However, Eden was statistically the same as Great Lake on the 3<sup>rd</sup> and 4<sup>th</sup> WAT. On the 5<sup>th</sup> WAT, the dry shoot weight of Omega and Eden were statistically the same but different from that of Great lakes. Biostimulant level also showed statistical differences among them (Table 4.6). fulvic humic acid concentration ( $B_2$ ) on the 1<sup>st</sup> WAT was statistically similar to seaweed humic concentration ( $B_1$ ). Thus, high in dry shoot weight but significantly different from the control ( $B_3$ ). Seaweed humic concentration ( $B_1$ ) obtained a high dry shoot weight from the 4<sup>th</sup> to the 5<sup>th</sup> WAT. While fulvic humic acid concentration ( $B_2$ ) recorded the lowest on the 4<sup>th</sup> WAT and  $B_3$  on the 5<sup>th</sup> WAT. However, fulvic humic acid concentration ( $B_2$ ) was statistically similar to fulvic humic acid concentration ( $B_2$ ) on both weeks. Seaweed humic concentration with eden variety ( $V1B1$ ) significantly recorded highest to the dry shoot weight in all WAT. While seaweed humic concentration with omega variety ( $V2B1$ ) was the least dry shoot weight on the 3<sup>rd</sup> WAT, fulvic humic acid concentration with great lakes variety ( $V3B2$ ) and no stimulant with Great lakes variety ( $V3B3$ ) on the 5<sup>th</sup> WAT (Table 4.6).

**Table 4.6 Bio stimulant and varietal effect on dry shoot weight of lettuce**

	Dry shoot weight (g)		
	3WAT	4WAT	5WAT
Variety			
Eden (V1)	0.98a	3.31a	4.92a
Omega (V2)	0.59b	1.99b	4.01a
Great lakes (V3)	0.64b	2.00b	2.96b
LSD (0.05)	0.18	0.50	0.92
P-value	<0.001	<0.001	<0.001
Bio stimulant			
Seaweed with humic (B1)	0.76a	2.76a	4.96a
Fulvic humic acid concentrate (B2)	0.92a	2.19b	3.62b
No stimulant (B3)	0.54b	2.53ab	3.31b
LSD (0.05)	0.18	0.50	0.92
P-value	<0.001	<0.001	<0.001
Variety x Bio stimulant			
V1×B1	1.13a	4.27a	6.81a
V2×B1	0.44c	2.16bcd	4.39b
V3×B1	0.71bc	1.84	3.69bc
V1×B2	1.13a	2.87b	4.57b
V2×B2	0.88ab	1.86d	3.69bc
V3×B2	0.74bc	1.86d	2.61c
V1×B3	0.68bc	1.84d	3.68bc
V2×B3	0.47c	1.97cd	3.95bc
V3×B3	0.47c	2.32bcd	2.60c
LSD (0.05)	0.31	0.87	1.61
P-value	<0.001	<0.001	<0.001
CV (%)	24.83	20.83	23.45

*Means followed by or sharing the same letters within a column are not significantly different at 5% level of significance, CV=coefficient of variation, LSD=least significant difference at 5%, WAT = Week after transplanting.*

#### **4.2.3 Fresh shoot weight**

Table 4.7 shows no significant ( $p>0.05$ ) difference between Eden and Omega variety of lettuce from week 3-5. There was also no significant difference between Omega variety and Great Lake from the 3<sup>rd</sup> to the 5<sup>th</sup> WAT. However, a significant ( $p<0.05$ ) difference was observed between Eden and Great Lake, with Eden variety weighting heavier than the other varieties along the weeks. The biostimulant level also showed no statistical differences on the 3<sup>rd</sup> and 5<sup>th</sup> WAT. On the 4<sup>th</sup> WAT, fulvic humic acid concentration (B2) and the control (B3) were statistically similar but significantly different from seaweed humic concentration (B1) which recorded the highest fresh root weight (Table 4.7). Interaction between eden variety (V<sub>1</sub>) and

seaweed humic concentration (B<sub>1</sub>) recorded a significantly higher in fresh shoot weight in all the weeks after transplanting while that of no stimulant with great lakes variety (V<sub>3</sub>B<sub>3</sub>) recorded the lowest fresh shoot weight for the 3<sup>rd</sup> and 5<sup>th</sup> WAT (Table 4.7).

**Table 4.7 Bio stimulant and varietal effect on fresh shoot weight of lettuce**

	Fresh shoot weight (g)		
	3WAT	4WAT	5WAT
<b>Variety</b>			
Eden (V1)	18.78a	27.01a	42.45a
Omega (V2)	16.44ab	24.19ab	38.86ab
Great lakes (V3)	13.89b	20.88b	31.36b
<b>LSD (0.05)</b>	3.51	4.42	8.48
P-value	<0.001	<0.001	<0.001
<b>Bio stimulant</b>			
Seaweed with humic (B1)	17.56a	27.86a	39.18a
Fulvic humic acid concentrate (B2)	17.00a	23.18b	39.63a
No stimulant (B3)	14.56a	21.04b	33.86a
<b>LSD (0.05)</b>	3.51	4.42	8.48
P-value	<0.001	<0.001	<0.001
<b>Variety x Bio stimulant</b>			
V1×B1	22.00a	34.41a	49.06a
V2×B1	17.33ab	25.62b	34.05bc
V3×B1	13.33b	23.55b	34.44abc
V1×B2	17.67ab	21.71bc	40.22ab
V2×B2	17.00ab	24.24b	43.52ab
V3×B2	16.33ab	23.61b	35.15abc
V1×B3	16.67ab	24.93b	38.07abc
V2×B3	15.00b	22.71bc	39.02abc
V3×B3	12.00b	115.48c	24.49c
<b>LSD (0.05)</b>	6.08	7.66	14.69
P-value	<0.001	<0.001	<0.001
<b>CV (%)</b>	21.46	18.42	22.61

*Means followed by or sharing the same letters within a column are not significantly different at 5% level of significance, CV=coefficient of variation, LSD=least significant difference at 5%, WAT = Week after transplanting.*

#### ***4.2.4 fresh root weight (g)***

Table 4.8 shows no significant ( $p>0.05$ ) difference among Eden, Omega and Great lakes variety of lettuce from 3-5WAT. Biostimulant level also showed no statistical differences among seaweed with humic (B1), fulvic humic acid (B2) and the control (B3) at 3WAT (seaweed humic concentration (B1) 1.00, fulvic humic acid concentration (B2) 1.11 and the control (B3) 1.22), 4WAT (seaweed humic concentration (B1) 1.74, fulvic humic concentration (B2) 1.58, and the control (B3) 1.94) and 5WAT (seaweed humic concentration (B1) 2.55, fulvic humic acid concentration (B2) 2.63, and the control (B3) 2.71) (Table 4.8). Interaction effect between varietal biostimulant also not significant on the 3<sup>rd</sup> WAT. Fulvic humic acid concentration with omega variety ( $V_2B_2$ ) recorded a significantly higher fresh root weight on the 5<sup>th</sup> week after transplanting. While no stimulant with omega variety ( $V_2B_3$ ) was the highest in the 4<sup>th</sup> WAT, no stimulant with great lakes ( $V_3B_3$ ) recorded a significantly lower fresh root weight on the 4<sup>th</sup> WAT while fulvic humic acid concentration with eden variety ( $V_1B_2$ ) was the lowest on the 5<sup>th</sup> WAT.

**Table 4.8 Bio stimulant and varietal effect on fresh root weight of lettuce**

	Fresh root weight (g)		
	3WAT	4WAT	5WAT
Variety			
Eden (V1)	1.11a	1.84a	2.65a
Omega (V2)	1.22a	1.92a	2.81a
Great lakes (V3)	1.00a	0.53a	2.43a
LSD (0.05)	0.35	0.43	0.65
P-value	<0.001	<0.001	<0.001
Bio stimulant			
Seaweed with humic (B1)	1.00a	1.74a	2.55a
Fulvic humic acid concentrate (B2)	1.11a	1.58a	2.63a
No stimulant (B3)	1.22a	1.97a	2.71a
LSD (0.05)	0.35	0.43	0.65
P-value	<0.001	<0.001	<0.001
Variety x Bio stimulant			
V1×B1	1.00a	2.12ab	3.08a
V2×B1	1.00a	1.46b	2.19ab
V3×B1	1.00a	1.64b	2.38ab
V1×B2	1.00a	1.47b	1.93b
V2×B2	1.33a	1.81ab	3.33a
V3×B2	1.00a	1.46b	2.63ab
V1×B3	1.33a	1.94ab	2.93ab
V2×B3	1.33a	2.48a	2.91ab
V3×B3	1.00a	1.49b	2.27ab
LSD (0.05)	<b>31.82</b>	<b>24.78</b>	<b>24.99</b>
P-value	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
CV (%)			

*Means followed by or sharing the same letters within a column are not significantly different at 5% level of significance, CV=coefficient of variation, LSD=least significant difference at 5%, WAT = Week after transplanting.*

## CHAPTER FIVE: DISCUSSION

### 5.1 Effect of Biostimulant and Variety on Growth Performance of Lettuce

The application of biostimulants, as observed in this study, promoted the growth parameters of lettuce when compared to the control plots. This effect can be attributed to the role biostimulants play in stimulating shoot elongation, leaf nutrient accumulation, and chlorophyll biosynthesis, which are critical for plant growth. According to Colla *et al.* (2015), biostimulants enhance nutrient uptake and shoot elongation, which explains why lettuce plants treated with biostimulants outperformed the untreated ones. Biostimulants stimulate essential physiological processes in plants, such as cell division and the biosynthesis of growth-related compounds, making them crucial for improving plant vigor and productivity.

A similar observation was made by Abdel Latef *et al.* (2017), who found that Indian pea plants treated with seaweed extracts exhibited significantly more vegetative growth compared to control plants. This suggests that the use of biostimulants like seaweed extracts can universally enhance plant growth. Additionally, it has been suggested that even when soil fertility is similar in both treated and untreated plots, biostimulants improve nutrient uptake (de Saeger *et al.*, 2020). They facilitate the uptake of nitrogen, prevent soil fixation of critical nutrients like phosphorus and potassium, and promote the formation of soil aggregates, which ultimately improves the soil's physical and chemical properties (Izosimov, 2016).

Moreover, biostimulants act as soil conditioners, enhancing soil quality. By improving the soil's nutrient availability and structure, biostimulants contribute to better plant growth (Kapoor *et al.*, 2021; Xu *et al.*, 2019). This improvement in soil properties, coupled with

the increase in nutrient uptake, likely contributed to the superior performance of biostimulant-treated lettuce plants.

The findings from this study are consistent with previous research. Hernandez *et al.* (2014) reported that the application of biostimulants led to an increase in leaf production, corroborating the higher leaf length observed at canopy spread in the biostimulant-treated lettuce. This is in agreement with the findings of Colla and Rouphael (2015) and Petropoulos (2020), as well as De Pascale *et al.* (2018), who also noted that biostimulants can promote leaf growth and overall plant development. The positive influence of seaweed extracts on growth parameters may be linked to the high cytokinin content found in seaweed, which promotes cell division and subsequently accelerates plant growth (Thomas *et al.*, 2013).

Dudaš *et al.* (2016) supported these findings by demonstrating that biostimulants such as Bio-algeen S-90 improved the growth of vegetables, including root and shoot weight, leaf length, and dry matter accumulation. In addition, Di Mola *et al.* (2020) reported that the use of seaweed extracts significantly enhanced the yield and leaf area index in baby leaf lettuce grown under greenhouse conditions, further validating the positive impact of biostimulants on plant growth.

In terms of varietal performance, the Eden variety outperformed the other lettuce varieties measured in this study. This superior performance may be due to the genetic composition of the Eden variety, which might be better suited to the environmental conditions in the study area, including soil fertility and climatic factors.

## 5.2 Effect of Biostimulant and Variety on Dry Matter Accumulation of Lettuce

The application of seaweed and humic acid biostimulants led to enhanced fresh shoot weight in lettuce, reflecting the impact of these biostimulants on plant growth and development. Chanthini *et al.* (2019) reported similar results, stating that seaweed biostimulants enhance root growth, which in turn promotes vegetative growth. This aligns with the current study's findings, where the higher fresh shoot weight in biostimulant-treated lettuce demonstrates the effectiveness of these natural growth enhancers.

The increase in shoot weight following biostimulant application is consistent with the work of Fan *et al.* (2014), Sible *et al.* (2021), and Abdulrahman (2013), all of whom reported that seaweed and humic acid biostimulants significantly boost shoot growth. Silicon, another effective biostimulant, stimulates plant growth through mechanisms such as enhanced cell division and elongation, better water regulation, and an overall boost to the plant's immune system (Na & Jiashu, 2001; Liang *et al.*, 2003). These effects are further supported by Goñi *et al.* (2018), who demonstrated that biostimulants like *Ascophyllum nodosum* extracts significantly increased dry matter in tomato plants even under irrigation stress, indicating their positive influence on biomass accumulation.

Biostimulants also have the ability to alter enzyme pathways and physiological mechanisms, leading to increased production of secondary metabolites such as carotenoids and polyphenols (De Sousa Oliveira *et al.*, 2013). These compounds enhance plant-fungal symbiosis, improve nutrient uptake, and increase the antioxidant activity of the plant. The findings of Basavaraja *et al.* (2018) support the observation that seaweed treatments stimulate growth hormone biosynthesis, leading to increased phenolic content and greater antioxidant capacity in plants. This is corroborated by the results of Ashour *et al.* (2021)

in studies on red hot peppers, where biostimulants promoted phenolic accumulation by improving growth and nutrient uptake.

In lettuce, the use of biostimulants combined with fertilizers has been shown to improve not only growth but also biochemical concentrations compared to untreated controls (Dudaš *et al.*, 2016). The findings of Khaled and Fawy (2011) further reinforce this, showing that humic and fulvic acids improve crop growth and yield in wheat. Interestingly, Sharif *et al.* (2002) found that low concentrations of fulvic acid significantly increased maize production, though higher concentrations led to diminished returns. This highlights the importance of optimizing biostimulant application for maximum growth benefits.

The positive effects of biostimulants on yield components such as 1000-grain weight, biological yield, and dry matter accumulation have been well documented, particularly in studies on wheat and other grain crops (Qin *et al.*, 2016; Tuba *et al.*, 2015). These studies suggest that biostimulants, when used correctly, have the potential to significantly enhance dry matter accumulation and overall plant performance, contributing to improved agricultural productivity and sustainability.

The application of biostimulants in this study demonstrated significant improvements in the growth performance and dry matter accumulation of lettuce. Seaweed extracts and humic acid proved to be highly effective in enhancing nutrient uptake, promoting shoot and root growth, and increasing phenolic compound accumulation. These results highlight the potential of biostimulants to improve the productivity and quality of lettuce and other crops, especially when combined with appropriate soil management practices.

## **CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS**

### **6.1 Conclusion**

The application of fulvic with humic acid (FH) and seaweed with humic (SH), significantly improved the number of leaves, leaf length, canopy spread, fresh and dry shoot weight, and fresh and dry root weight compared to the control. Seaweed with humic produced the mean value in all the growth parameters taken. Eden variety had the highest mean value of number of leaves, leaf length, canopy spread and dry matter accumulation, and the lowest was the Great lakes. The study found that interaction between seaweed humic concentration with Eden variety (V1B1) was the highest and that of no stimulant with great lakes variety (V3B3) was the lowest on dry matter accumulation. However, on the growth parameters, interaction between seaweed humic concentration with omega variety (V2B1) as the highest on leaf length and canopy and seaweed humic concentration with Eden variety (V1B1) on number of leaves.

### **6.2 Recommendations**

From findings the following: recommendation are made;

1. The use of Eden variety is recommended for large scale farming in the Mampong municipality.
2. Farmers in Asante Mampong interested in biostimulant should use Seaweed with humic combination.
3. Combined use of Eden variety and Seaweed with Humic biostimulant is encourage for maximum growth of lettuce.

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