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Assessing seaweed extract as a biostimulant on the yield of organic leafy greens in Tennessee[1,2]

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ABSTRACT

Biostimulants are a better alternative to expensive organic fertilizers used in certified organic crop production. Seaweed extracts (Stimplex®)[6], derived from brown algae *(Ascophyllum nodosum)* present in marine water, can be used as a "biostimulant" in agriculture to enhance plant yield and improve plant growth. The aim of this study was to elucidate the effect of Stimplex® on the yield of leafy green vegetables produced in an organic farming system. A Stimplex® greenhouse trial was conducted in fall 2015 and spring 2016 at the Tennessee State University organic research farm. Six leafy greens –lettuce, mustard, kale, Swiss chard, amaranths and collards– were foliar sprayed with Stimplex® (treated) bi-weekly for six weeks at the recommended dose of 3.2 ml/L (0.4 oz/gal of water), and six were untreated (control). Seedlings were raised in the greenhouse from organic seeds in pots, and organic management practices were followed per National Organic Program standards throughout the study. For each leafy green, a total of five treated potted plants and five untreated potted plants were used in three replications. Results showed that plants treated with Stimplex® were more vigorous, healthier and increased yield over untreated plants, except for amaranths. The percentage increase in plant yield of all crops was between 11% and 35%. The highest and lowest increase in plant yield was observed in the mustard greens (35.8 %) and Swiss chard (11.0%). Results suggest Stimplex® stimulates higher yields in leafy greens.

Key words: untreated environment, greenhouse production, seaweed extract, organic farming system, leafy green vegetables

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[6]Company or trade names in this publication are used only to provide specific information. Mention of a company or trade name does not constitute an endorsement by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

RESUMEN

Evaluación de un extracto de alga como un bioestimulante del rendimiento de vegetales de hoja orgánicos en Tennessee

Los bioestimulantes son una mejor alternativa a los costosos fertilizantes orgánicos utilizados en la producción de cultivos orgánicos certificados. Los extractos de algas marinas (Stimplex®), derivado de algas marrones *(Ascophyllum nodosum)* presente en el agua marina, pueden ser utilizados como un 'bioestimulante' en la agricultura para mejorar el rendimiento de las plantas y mejorar el crecimiento. El objetivo de este estudio fue elucidar el efecto de Stimplex® sobre el rendimiento de las hortalizas de hoja verde producidas en un sistema de cultivo orgánico. Un ensayo de invernadero se llevó a cabo en el otoño de 2015 y primavera de 2016 en la granja de investigación orgánica de la Universidad Estatal de Tennessee. Seis vegetales de hoja –lechuga, mostaza, col rizada, acelgas suizas, amaranto y berza verde– se asperjaron foliarmente con Stimplex® (Tratado) bisemanalmente por seis semanas a la dosis recomendada de 3.2 ml/L (0.4 onzas/galón de agua) y seis no tratados (Control). Las plántulas se produjeron en el invernadero a partir de semillas orgánicas en macetas, y a lo largo del estudio se siguieron las prácticas de manejo orgánico según las normas del programa nacional orgánico. Por cada vegetal de hoja se utilizaron cinco plantas en maceta tratadas y cinco plantas en maceta no tratadas con tres replicaciones. Los resultados mostraron que las plantas tratadas con Stimplex® eran más vigorosos, sanos y aumentaban el rendimiento sobre las plantas no tratadas, excepto los amarantos. El aumento porcentual en el rendimiento vegetal de todos los cultivos fue entre 11% y 35%. El aumento más alto y más bajo en el rendimiento de la planta se observó en las hojas de mostaza (35.8%) y acelgas suizas (11.0%). Los resultados sugieren que Stimplex® estimula mayores rendimientos en los vegetales de hoja.

Palabras clave: ambiente no tratado, spray foliar, producción de invernadero, extracto de algas marinas

INTRODUCTION

According to Yakhin et al. (2017), a biostimulant can be defined as "a formulated product that improves plant productivity by a mechanism of action that is not the sole consequence of the presence of known essential plant nutrients, plant hormones, plant growth regulators or plant protective compounds." Biostimulants can be used for various purposes such as improving nutrient use, helping plants tolerate abiotic stress (heat, cold, water logging, drought, etc.) and enhancing general quality attributes such as yield, appearance and nutritional content. In 2007, the term biostimulants was first used in scientific literature and defined as materials other than fertilizers that promote plant growth when applied in low quantities (Kauffman et al., 2007). Plant biostimulants can be directly incorporated into the soil or sprayed as foliar application on plants, and they influence the nutrient uptake, microbial activity in soil, plant growth and development by both means of application (Chen et al., 2003; Csizinszky, 1996). Over

the past few years, plant biostimulant industry sales have increased about $2 billion yearly, and the use of these products in agriculture also grew immensely (Calvo et al., 2014). According to Kauffman et al. (2007), biostimulants can be found in various formulations and are classified into three major classes, which include humic substances (HS), hormone containing products (HCP), and amino acid containing products (AACP). Among the hormone containing products are seaweed extracts which contain cytokinins, auxins and derivatives as active plant growth substances. In their study, Yakhin et al. (2017) classified a large list of biostimulants by origin–from seaweeds, microorganisms, food waste, insect chitin, higher plant parts, amino acids from animals and so on.

A large number of plant biostimulants are extracted from biological materials derived from biological tissue or cells that are used to enhance plant growth and yield (Sandhu and Nandwani, 2016). According to Food and Agriculture Organization (FAO) reports, 15 million metric tons of seaweed extract products are produced yearly and often used as biostimulants, plant growth enhancers and soil conditioners in agriculture (FAO, 2006). Seaweed extracts are also known as biological fertilizers (Zodape, 2001). *Ascophyllum nodosum* (L.), a brown algae seaweed species of marine origin, has been widely studied and used in various ways –as fertilizer, to condition the soil, as a source of animal feed and human nutrition supplement (Fan et al., 2011). A few reports have suggested that biostimulants derived from brown algae have been used by farmers since the 12th century (Temple and Bomke, 1988). Although seaweeds have been used in agriculture since ancient times, their function as a biostimulant was discovered only in the last few decades. The active ingredients of seaweeds have been extracted and sold commercially in concentrated form. Application of seaweed extracts can be made through foliar sprays, root dips or soil drenches. Mooney et al. (1986) reported that seaweed extracts contain cytokinins, auxins, vitamins, amino acids, essential and non-essential plant nutrients, and abscisic acid (ABA) growth substances.

Organic farming is growing fast and gaining popularity worldwide (Nandwani and Nwosisi, 2016). Leafy green vegetables are crops with leaves that are consumed daily in many parts of the world, and crops that fall into this category belong to families that include Brassicaceae, Asteraceae, Chenopodiaceae, etc. (Kaiser and Ernst, 2012). Most leafy greens are cool season crops; however, with prolonged winters in temperate regions, greenhouses can be used to protect the crop from cold and extend the growing season (Kaiser and Ernst, 2012). As the demand for organic and locally grown food rises, consumer preference for a wider variety of fresh food products favors leafy green vegetable pro-

duction. There is a need to improve quality and yield in organic leafy greens to meet the growing demand of the organic leafy greens market.

Natural products can be used as biostimulants in vegetable production, even though their mechanism of action is not certain. Seaweeds and their derivatives are approved for use as organic fertilizers in the Annex I of the Reg. (EC) No. 889/2008, even though their properties as a biostimulant have not yet been elucidated (Alessandra et al., 2014).

Since there are not many organic fertilizer options available in organic farming, Stimplex® may serve as an alternative. Akande (2006) compared the effect of an organic root biostimulant with a conventional fertilizer on the growth of amaranth and found that the biostimulant was more effective when combined with a mineral fertilizer. As growth promoters, biostimulants are relatively novel products in organic agriculture, and Stimplex® is one of the most popular in organic farming. Stimplex® is a seaweed extract used as a biostimulant known to have a complimentary effect on yield, growth and plant development. Stimplex® contains 0.01% cytokinin and 99.99% other unknown ingredients in brown algae (*Ascophyllum nodosum)*, present in marine water.

Several studies have reported that yields of various crops increased significantly as a result of seaweed extract application by the foliar method (Arthur et al., 2003; Zodape et al., 2008). In organic farming systems, several studies have reported that Stimplex® increased yields of vegetable crops and improved quality of fruits (Fornes et al., 1995 and 2002; Koo and Mayo, 1994; Norrie et al., 2002). Metting et al. (1990) reported that seaweed extracts showed many positive results on leafy vegetables including Swiss chard, brassicas and peppers. These extracts promote shoot growth (Atzmon and Van Staden, 1994) and enhance the development of roots. Higher levels of organic matter have been found in seaweed fertilizers than in chemically derived fertilizers, which help in retaining minerals and moisture in the upper parts of the soil for root availability (Sivasankari et al., 2006). According to Crouch and Van Staden (1993) the positive influences noted in various crops treated with seaweed extracts are due to the presence of cytokinin.

Many studies compare the nutritional levels of organically grown vegetables to those in conventional farming systems. Though not much research has been done on the mineral content of organic vegetables treated with biostimulants, in the foreseeable future further studies will be required.

The negative effects of chemical fertilizers have led more growers to replace them with organic fertilizers (Bhatia, 2002). Using seaweed extracts as plant biostimulants (Zodape, 2001) would help meet increasing demand. Because various chemical fertilizers are prohibited in organic farming, and few organic fertilizers are available, mostly

slow releasing fertilizers like blood meal and poultry manure, biostimulants could serve as an alternative for organic growers to increase their yields and possibly profits. With all the evidence of positive results of seaweed extracts on plant growth and yield in previous literature, we hypothesize that Stimplex® will increase the yield of organic leafy green vegetables. We hypothesized that the plants of six vegetables treated with Stimplex® will have higher yields than the untreated plants. The main objective of this study was to determine the effect of Stimplex® on the yield of six organic leafy green vegetables.

MATERIALS AND METHODS

Greenhouse experiments were done in fall 2015 and spring 2016 at the organic research farm (latitude 36° 10′ N and longitude 86° 49′ W) at Tennessee State University, Nashville, TN. Sandy loam soil with good drainage was collected from the Tennessee State University certified organic research farm, Nashville, TN, USA. The soil report (Northeastern University, Boston, MA) indicated that the soil in the experimental area contained 5.80% organic matter, 2.65% humic acid and 0.32% fumaric acid, essential compounds that enable a combination of minerals to be made available to plants. On average, temperatures of 22 to 24 °C (72 to 75 °F) were maintained inside the greenhouse daily.

Organic seeds of six leafy green vegetables were procured from Territorial Seed Company, Johnny Seeds and Sow True Seeds. 'Coastal Star', 'White Russian', 'Giant Red', 'Champion', 'Hopi Red Dye' and 'Rainbow Mix' were the commercially available varieties used in the experiment for lettuce, kale, mustard greens, collards, amaranths and Swiss chard, respectively. For each leafy green type, plants were direct seeded into five 1 L plastic pots (Hummert International, MO). There were six rows (one row of each leafy green type) with five potted plants in each row; a total of 15 plants for each leafy green were assigned to untreated (no Stimplex®) and Stimplex® treated plants. Plastic pots were filled with an organic transplant mix consisting of soil, farmer produced compost (Nature Safe, Irving, TX) and peat moss media (Hummert International, MO) in a 4:1:1 ratio, respectively. The pots were arranged on ground covered with plastic sheet in the greenhouse and three replications were used for the study. All the plants were direct seeded into the pots, thinned when seedlings were 15 days old, and one seedling per pot was kept for the experiment and the remaining plants were discarded from the pot.

An equal dose of 3.2 ml/L (0.4 oz/gal) of Stimplex® (Anacardian) was applied to all treated plants, which were foliar sprayed, as recommended by the manufacturing company, while untreated plants were

sprayed with water. The first application of Stimplex® (Acadian Seaplants Ltd, Canada) was foliar sprayed to young seedlings 10 days after thinning. Consecutive sprays of Stimplex® were applied at 14-day intervals for a period of six weeks. During the 6-week growing period, the plants were irrigated manually, as needed. Organic management practices were followed as per National Organic Program (NOP) standards. Organic living fertilizer (Mighty grow; 4:3:4) was applied at a rate of 560 kg/ha (500 lb/a) for nutrient management.

Both untreated and treated plants of all crops were harvested manually with harvesting knives at the end of the crop season. A second harvest was conducted for those crops (kale, mustard, collards) where usual production practices take into account multiple harvests. All crop plants were assessed during the trial for overall appearance and robustness. Data were collected on plant weight from all plants/replications, for a total of 15 plants (treated and untreated) with five plants per replication. Leaf lamina was measured (in cm) using a Vernier caliper. Data were analyzed using SAS software (version 9.4) in triplicate. An independent t-test was used to determine the significant differences in yield performance, and means ($p<0.05$) were compared between the various leafy green vegetables (treated and untreated).

RESULTS AND DISCUSSION

All crop yields responded positively (Figure 1) to the Stimplex® treatment given to the plants. Lettuce plants treated with Stimplex® had significantly ($p<0.05$) more weight than did the untreated plants. Untreated lettuce and Stimplex® treated plants had average per plant weight of 262.27 and 343.37 g, respectively. In a field trial study by Amanda et al. (2009), under plastic tunnel conditions, lettuce treated with a biostimulant (Actiwave) showed increased yields, chlorophyll, carotenoid, total phenols and antioxidant content but lower leaf nitrate content (likely due to environmental conditions) than in the untreated lettuce. Alessandra et al. (2014) evaluated the effect of two diluted doses of a biostimulant from filtrate seaweed extract on a greenhouse pot trial of organic lettuce and discovered an increase in dry weight and nutrient uptake of plants in the biostimulant treated lettuce, which showed greater efficiency relative to P and K macro elements; however, efficiency was greatest at the highest dilutions.

In mustard, Stimplex® treated plants weighed significantly more (500.77 g) when compared to the untreated (368.63 g). Leaves of Stimplex®-treated mustard plants appeared to be smoother, dark-colored and portrayed more red coloration on their leaves in comparison to the untreated plants. Collard, Swiss chard and kale plants treated

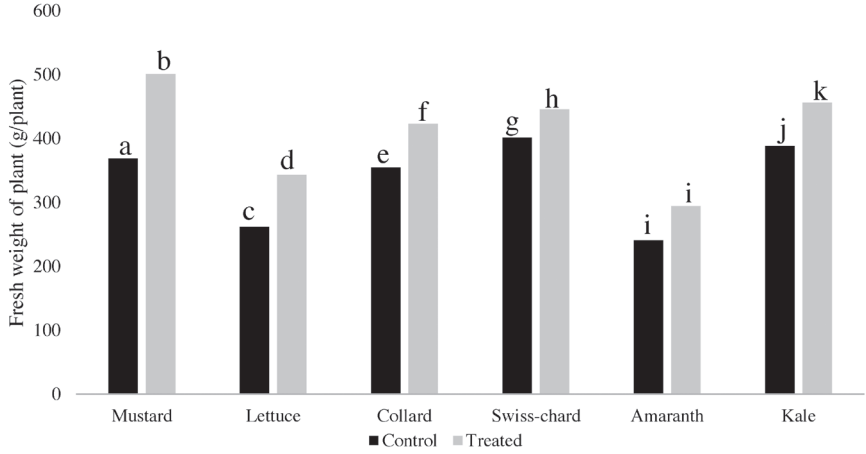

FIGURE 1. Weight of leafy green vegetable plants untreated and treated with Stimplex®
NOTE: MEANS WITH THE SAME LETTERS ARE NOT SIGNIFICANTLY DIFFERENT.

with Stimplex® showed a significant increase in weight compared to the untreated plants. Weight of the untreated collard, Swiss chard and kale plants were 354.43 g, 401.33 g and 388.40 g, respectively. Stimplex® treated collard, Swiss chard and kale plants on the other hand, weighed 423.00 g, 445.70 g and 456.20 g, respectively.

In contrast, amaranth plants treated with Stimplex® did not show any significant difference in weight when compared with the untreated plants. Average per plant weight of the untreated and treated amaranth plants was 240.67 g and 294.47 g, respectively. The plants treated with Stimplex®, except for collards, showed a significant increase in leaf lamina (Table 1.).

According to Scaglia et al. (2017), biostimulants improve plant growth by stimulating nutrient uptake and efficiency, improving tolerance to abiotic stress and increasing crop quality. Jayaraman et al. (2010) reported that plants treated with the biostimulant Stimplex® showed reduced incidence of diseases and other pathogens of disease. In addition, biostimulants help improve the nutrient uptake through the roots and leaves (Vernieri et al., 2005; Mancuso et al., 2006) which could lead to enhanced plant growth and vigor. Several studies have reported that biostimulants significantly increase plant production (Popescu, 2013). Norrie and Keathley (2005) reported the ability of *Ascophyllum nodusum* to enhance grape yield. In a study by Popescu and Popescu (2014), foliar applications of three concentrations of seaweed extracts were used to determine their effects on the growth of grapevine with positive results that showed increase in growth at

TABLE 1.—*Leaf lamina (in cm) of green vegetable plants untreated and treated with Stimplex®.*

Leafy Green	Untreated	Treated
Mustard	22.50 a[1]	29.30 b
Kale	14.20 c	18.90 d
Collard	12.50 e	15.30 e
Swiss chard	14.75 f	21.30 g

[1]Means in a row with the same letters are not significantly different.

higher concentrations of seaweed extracts. Dominguez and Robinson (2015) reported that biostimulants improved yields in different cultivars of apple trees compared to the untreated plantings. A research study by Nelson and van Staden (1984) reported that seaweed extracts (Kelpak 66) increased the yield of bean plants by 24% when foliarly applied. Popescu (2013) discovered that soil and foliar application of the brown algae seaweed extract led to a significant growth of bean and mustard plants. Crouch and van Staden (1992) conducted a study on tomatoes in which they observed improved quality and a 30% increase in the fresh weight of fruits sprayed with seaweed extracts at an early stage of vegetative growth.

Although seaweed products are becoming very popular in agriculture to increase yields and plant growth, nevertheless the exact mode of action is still unknown (Khan et al., 2009). Biostimulants are believed to consist of hormonal substances, such as cytokinins and auxins (Stirk et al., 2003; Khan et al., 2009; Craigie, 2011), which may be associated with the increased yield of treated plants when compared to the untreated plants (Featonby-Smith and van Staden, 1983 and 1984; Khan et al., 2009; Craigie, 2011). Furthermore, several studies have reported that seaweed extracts increase the levels of certain compounds in plants, such as betines, antioxidants, cytokinins and proline that are responsible for increased tolerance to stress (Zhang and Ervin, 2004, 2008; Zhang et al., 2010; Aziz et al., 2011; Lola-Luz et al., 2013; Fan et al., 2013).

Treatment of plants with seaweed extracts may increase chlorophyll content in plant leaves due to a decrease in chlorophyll breakdown (Blunden et al., 1996) and may also increase the populations of soil microorganisms leading to better root growth (Calvo et al., 2014). Fan et al. (2013) reported an increase in antioxidant levels, soluble proteins and polyphenolic content of spinach treated with the brown algae seaweed extract. Calvo et al. (2014) reported the resulting increase in such nutritional elements in the plant to be synergistic with specific enzymes responsible for nitrogen, glycine betaine and antioxidant syn-

thesis. Increased protein levels as reported by Abbas (2013) may be due to increased concentrations of carbohydrates in plant leaves.

From a biochemical standpoint, humic substances elucidate a positive effect on plant growth through their potential to improve the structure and fertility of the soil, thereby building up the soil and enhancing the ability of the plant to take up nutrients (Trevisan et al., 2010). Aisha et al. (2014) reported that increasing levels of humic acid and organic compost manure fertilizer on the turnip mustard lead to a higher percentage of nutritional elements such as carbohydrates, N, P, K, Zn, Fe and Mn in the roots and an increase in plant growth. Naturally occurring biostimulants such as humic substances regulate carbon and nitrogen breakdown, increasing the enzymes that function in Krebs' cycle, nitrate assimilation and glycolysis (Nardi et al., 2009; Ertani et al., 2013a; Ertani et al., 2013b; Ertani et al., 2014; Canellas and Olivares, 2014). In a study by Pizzeghello et al. (2013), indole acetic acid was described as the bioactive compound present in humic substances, and when the cytokinin-like activity of humic substances was measured in small radish leaves, increased levels were found in humic substances obtained from the excreta of earthworm (Pizzeghello et al., 2013). Several studies have also reported the ability of fulvic acid to improve nutrient uptake in a myriad of systems (Calvo et al., 2014).

All the formerly mentioned studies show that Stimplex®, the biostimulant from brown algae seaweed extract, may have a synergistic effect on one or more bioactive compounds present in the soil or plants that may positively influence plant growth. However, these compounds and their properties need to be identified, and further research is required to explain their corresponding properties. To ensure environmental sustainability, Stimplex® could serve as a natural product option to the number of chemical plant growth regulators available today and provide a means of increasing the weight of organic leafy green vegetables.

CONCLUSION

From the results we concluded that Stimplex® can be used as a biostimulant to enhance plant growth and yield of leafy green vegetable plants. All Stimplex®-treated plants showed an increase in average per plant weight except for the amaranth. Of all the leafy green vegetables tested, the 'Giant Red' mustard variety grown under greenhouse controlled conditions of the certified organic farm had the best response to the foliar application of Stimplex® (*Ascophyllum nodosum* extract) at a similar concentration of 3.2 ml/L (0.4 oz/gal). The results may be due to individual action or synergistic effect of one or more of

the compounds present in Stimplex® on the growth of the plants. More research on biostimulants from agronomic to molecular studies is required to determine the action mechanism of its biological processes. Further studies on different concentration deliveries, dose optimization and application methods of Stimplex® need to be done to make further recommendations.

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