



The Effect of Seaweed Extract (*Sargassum Sp*) Used as Fertilizer on Plant Growth of *Capsicum Annum* (Chilli) and *Lycopersicon Esculentum* (Tomato)

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ABSTRACT

Seaweeds, mainly brown species, have long been used as soil fertilizers and have several advantageous effects of spraying their crude extracts on plant growth. Unlike chemical fertilizer, biofertilizers derived from seaweeds or other organisms are usually biodegradable, non-toxic, non-polluting and non-hazardous to humans, animals and birds which will benefit to agriculture sector. In this study, four different concentrations (0.50; 1.00; 2.50; and 5.00 mg/L) of four different solvents extract (hexane, dichloromethane, methanol and water) were used to determine the effect on *in vitro* seed germination of *Capsicum annum* and *Lycopersicon esculentum*. The results showed that concentration 2.50 mg/L can be classified as optimum concentration for seedling development of *Lycopersicon esculentum* and this study has proven that DCM is the effective extract solvent that result in promoting hypocotyl and root growth of tomato plant. For *Capsicum annum*, hexane extracts solvent of *Sargassum sp.* Was found to be more effective than other extracts in promoting seedlings development. Phytochemical screening of *Sargassum sp.* exhibited the presence of alkaloids and terpenoids as the dominant compenants in all extract.

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1. INTRODUCTION

Crude extracts from seaweeds have been shown to exhibit many bioactivities that include biostimulant, fertilizer and antimicrobial properties and have been used to improve the growth of plants. As a result of emphasis upon the need to adopt eco-friendly agricultural practices for sustainable agriculture, there has been a high demand for organic farm products by the health society and attempts are now being made by farmers across the globe to detoxify the land by switching over to organic farming methods (Sangeetha & Thevanathan, 2010). This is because the extensive application of chemical fertilizers to increase crop productivity has led to impairment to the ecology of agricultural systems and has also reduced the nutritional quality of various crops (Kumari et al., 2011).

Sargassum species has been used as biofertilizers to enhance plant growth and yield and still need to be evaluated not only as rich sources of mineral nutrients but also for the growth promoting substance they possess (Sivasankari et al., 2006). Several seaweed species have been identified for their considerable economic importance such as food for humans, industrial materials, ingredients in traditional medicine, and as biofertilizers (Hong et al., 2007). Hong et al., (2007) further indicate that seaweeds have high quality materials for industrial and agricultural purposes.

Tomato is one of the most important vegetables in the world, which ranks second after potato in annual farm production. It contains a rich source of minerals, carbohydrates, fats, proteins, vitamins, lycopene, α and β carotene, lutein, zeaxanthin, and β -cryptoxanthin (Kumari et al., 2011). Vitamin C (ascorbic acid), while being an effective antioxidant in plants, is an important phytochemical constituent of tomato fruit (Kumari et al., 2011).

Also, tomatoes have low caloric value, high fiber content and phenols such as flavonoids, hence they are considered as excellent fruit vegetable that provides physiological and nutritional benefits (Dorais et al., 2008). Studies have shown strong inverse correlations between the consumption of tomato and the risk of certain types of cancer, cardiovascular diseases and age-related macular degeneration (Dorais et al., 2008).

Similarly, Chilli (*Capsicum annum L.*) is one of the most essential spicy vegetable and condiments crop having immense commercial dietary and therapeutic values (Meena et al., 2013), which is commonly grown by farmers during dry climatic season especially because of its high economic returns and export potential (Jayasinghe et al., 2016). Chilli belongs to the family Solanaceae, which refers to fruit that is usually cone-shaped, smaller and the pungency may range from being very mild to intensely hot (Chowdhury et al., 2015). Pepper is widely cultivated for its fruits which have a recognized nutritional value. In fact, they are an excellent source of various antioxidant compounds like flavonoids, carotenoids and Vitamin-C (Chuah et al., 2008).

In previous studies, the application of *Sargassum sp* to *Capsicum annum* resulted in significant increase of several growth parameters such as shoot weight, root weight, number of leaves and number of pods when compared to the extracts preparation from other species (Jayasinghe et al., 2016). Previously, we conducted experiments on tomato to study the potential of brown alga (*Sargassum sp*) as a biofertilizer. At higher concentrations of seaweed extract application, results recorded a statistically significant increase in the vegetative growth of tomato plant parts (height, shoot length, root length, as well as number of branches), reproductive parameters such as flower number, fruit number, and fresh weight, and biochemical constituents which includes the photosynthetic pigments,

proteins, total soluble sugars, reducing sugars, starch, phenols, lycopene, and vitamin C (Kumari *et al.*, 2011, Martin & Rahmat, 2017). Thus, current study is conducted to determine the effect of different *Sargassum sp* concentration on *in vitro* seed germination of *Capsicum annum* and *Lycopersicon esculentum* and also to carry out phytochemical screening analysis of *Sargassum sp*.

2. MATERIALS AND METHODS

2.1. Extraction of *Sargassum sp*

Seaweed *Sargassum sp.* was collected from Semporna, Sabah. Then, the fresh seaweed was weighed to get the wet weight before drying it in the oven for three to four days. After the drying process, *Sargassum sp.* was weighed again to get the dry weight. The sample was soaked into four different solvents which are methanol, hexane, dichloromethane and water for three to five days. In order to get the crude extract, rotavape process was used using rotary evaporator. The obtained crude extract was put in the beaker and covered it using the aluminium foil by making small holes on the top for it to evaporate.

2.2. Phytochemical screening analysis

The purpose of this phytochemical screening analysis was conducted to test the presence of alkaloids, terpenoids, tannins, saponins and flavonoids. To carry out this analysis, stock solution was prepared first by diluting the 1 g in 100 mL of crude extract with their mother solvent. The qualitative results were expressed as (+) for the presence and (-) for the absence of phytochemicals.

2.3. Media preparation

Firstly, 500 mL of sterilized distilled water was added with 25 mL of macronutrient and 2.5 mL of each of the following nutrient; micronutrient, iron and vitamin. Then, the solution was checked for pH = 5.8. 8 g of agar, 15 g of sucrose, and 0.05 g/L of Myo-inositol was added into that solution. All the extract stock

solution with different concentrations (0.50; 1.00; 2.50; and 5.00 mg/L) then were injected using micropipette in MSO agar medium. All the mediums were autoclaved to prevent contamination.

2.4. Surface sterilization of seed

The seeds were rinsed thoroughly under running tap water for 10-30 minutes and then 3 times with distilled water. The seeds were soaked in a mixture of 100% sodium hypochlorite and 1-2 drops of Tween-20 for minutes, followed by 75, 50, 30, and 20% of sodium hypochlorite. Then, each treatment was shaken for 2-3 minutes. The seeds then were rinsed at least 3 times with sterile distilled water in the Laminar Flow chamber equipped with ultra violet light.

2.5. In-Vitro seed germination

All aseptic work was carried out in the laminar flow chamber. The sterile seeds were placed on the sterile filter paper and allow to dry. Then, two sterile seeds was inoculated on the MS medium (MSO, MS + *Sargassum* crude extract (0.50; 1.00; 2.50; and 5.00 mg/L). All cultures then were maintained in the culture room at 25°C with 16 hour light and 8 hours dark photoperiod. The culture was observed alternately for 4 weeks. Lastly, the lengths of hypocotyle and roots of *Capsicum annum* and *Lycopersicon esculentum* were measured.

3. RESULTS AND DISCUSSION

3.1. Phytochemical screening analysis

The phytochemical screening of *Sargassum sp.* showed the occurrence of possible bioactive components in different extracts. The qualitative phytochemical analysis exhibited the presence of alkaloids, terpenoids, tannins and saponins in *Sargassum sp.* from all four solvents used to extract the macroalgae. The data were tabulated in Table 1. Dichloromethane, a polar solvent was found to con-

tain the most chemical constituents in the extraction, while methanol and hexane solvents were able to extract alkaloids and terpenoid with moderate concentrations. In tannins test, the higher color intensity was observed in dichloromethane extract of *Sargassum sp.* followed by hexane and water extract that have low color intensity but no reaction occurred in methanol. DCM and methanol can be classified as the better solvent for extracting saponins from the macroalgae as both showed reaction although at low intensity compared to hexane and water.

The phytochemical qualitative results showed that terpenoids and alkaloids were the dominant components in all extract. A wide range of solvent has been proven to be effective in extracting to extract alkaloids

from macroalgae. For example, ethanolic solvent was found to efficiently extract flavonoid from macroalgae class Phaeophyceae and moderately in class Chlorophyceae and Rhodophyceae (Sarojini *et al.*, 2012). Another study has also shown hexane could effectively extract flavonoids from *Kappaphycus alvarezii*, a member of red seaweed, followed by chloroform, ethyl acetate and methanol (Lalopua *et al.*, 2011), which is in contrast to our findings. A study carried out by Siregar *et al.*, (2012) demonstrated that the efficiency of hexane and methanol to extract alkaloid and flavonoid compounds from genus *Sargassum* but for our study, we found that DCM and hexane are the most efficient solvents to extract alkaloids and terpenoids from the macroalgae studied.

Tabel 1. The qualitative screening phytochemical screening results of *Sargassum sp*

Solution	Alkaloids	Terpenoids	Tannins	Saponins	Flavonoids
DCM	+++	+++	++	+	-
Methanol	+	+	-	+	-
Hexane	++	++	+	-	-
Water	-	++	+	-	-

“-: Not Found; +: Low Concentration; ++: Moderate Concentration; +++: High Concentration”

3.2. Seeding development of *Lycopersicon esculentum*

Figures 1-6 showed the quantitative parameters of the seedling development of *Lycopersicon esculentum*. In this study, the researcher focused on a few main parameters which are plant height, stem height, root height, stem weight, stem fresh weight and the total fresh weight to show tomato plant development. The result above showed the development of measured parameter when inoculated in four different concentration of crude extract which are 0.50; 1.00; 2.50; and 5.00 mg/L.

For *Lycopersicon esculentum*, we found that concentration 2.50 mg/L can be classified as optimum concentration for seedling development. This is because, most of the crude solvents showed the positive development in 2.50 mg/L when compared with MSO. For example, Figure 1 indicates that tomato plant have high potential to grow in 2.50 mg/L as they showed increment for all solvents extract except methanol which has lower mean of plant height when comparing with MSO.

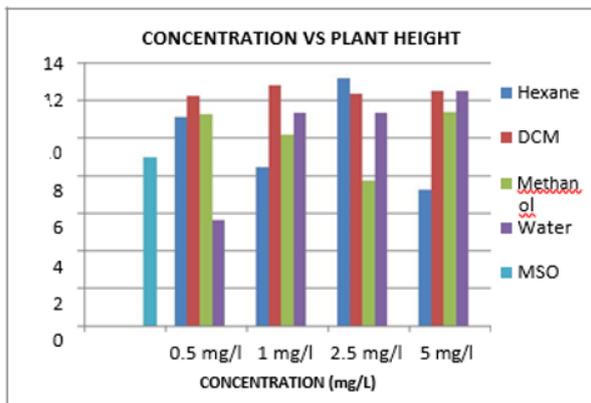


Figure 1. Concentration vs plant height

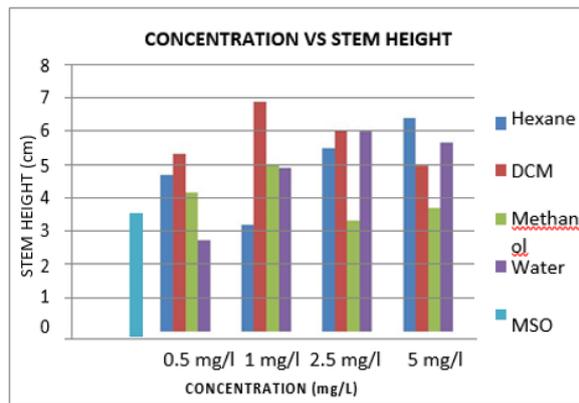


Figure 2. Concentration vs stem height

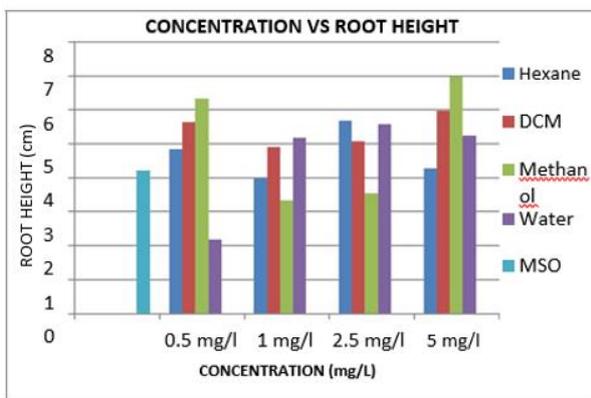


Figure 3. Concentration vs root height

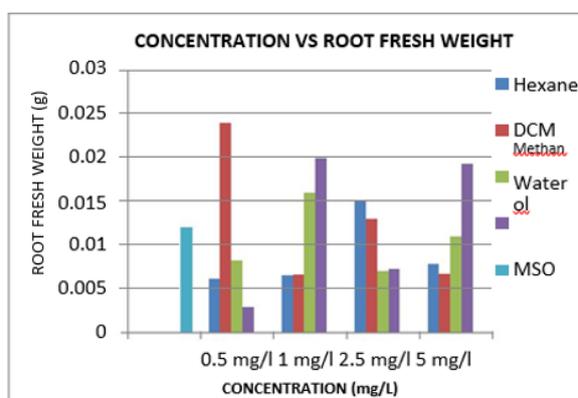


Figure 4. Concentration vs root fresh weight

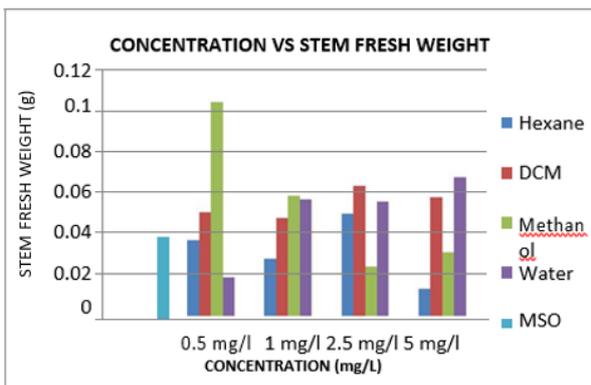


Figure 5. Concentration vs stem fresh weight

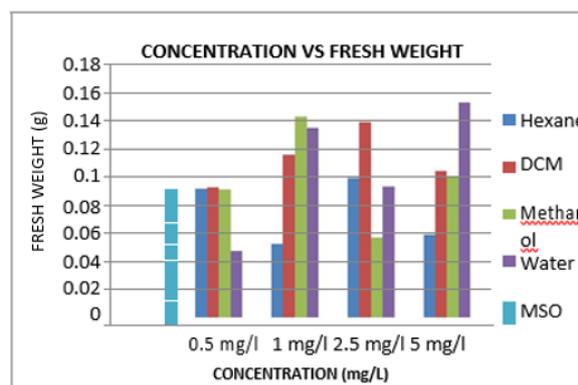


Figure 6. Concentration vs fresh weight

Previously, some researchers conducted experiments on tomato to study the potential of brown alga (*Sargassum sp*) as a biofertilizer. At higher concentrations of seaweed extract application, results recorded a statistically significant increase in the vegetative growth of tomato plant parts (height, shoot length, root length, as well as number of branches), reproductive parameters such as flower number, fruit number, and fresh weight, and biochemical constituents which includes the photosynthetic pigments, proteins, total soluble sugars, reducing sugars, starch, phenols, lycopene, and vitamin C (Kumari et al., 2011).

For tomato plant, based on quantitative data obtained (See Figures 7 and 8), we found that the most effective extract solvent that result in promoting hypocotyl and root growth of tomato plant was dichloromethane as DCM showed positive increment for every main parameters that has been measured. For this study, dichloromethane can be the most potential of *Sargassum* extracts in enhancing the seed germination and seedlings development especially for *Lycopersicon esculentum*.



Figure 7. Effect of additional chemicals: (left) MSO fresh weight, (middle) 2.50 mg/L of DCM, and (right) 2.50 mg/L of hexane



Figure 8. Effect of additional chemicals: (left) 2.50 mg/L of water and (right) 2.50 mg/L of methanol

3.3. Turpis egestas

Figures 9-14 showed the quantitative parameters of the seedling development of *Capsicum annum*. The effects of *Sargassum sp.* extract obtained on growth of *Capsicum annum* have shown interesting results based on positive development that focus on a few main parameters which are plant height, stem height, root height, stem weight, stem fresh weight and the total fresh weight. Previous study has proven some findings that the application of *Sargassum wightii* liquid extracts has increased seed germination percentage and growth of Triticum (Kumar & Sahoo, 2011). In other research, Sridhar and Rengasamy, (2012) found that the aqueous extracts of *S. wightii* was found to promote the *Capsicum annum* growth and yield at concentration of 1.00%.

In our study, Figure 9-14 showed the data obtained that have been compared with four different concentration from four different extract solvents. For Figure 9, 10, 11, and 12, data obtained obviously showed the increment for every concentration from four extract solvents when comparing to MSO as a control solvent extract. Besides that, the

other parameters that have been plotted in Figures 13 and 14 also indicated the positive development mostly for all concentration from four extract solvents, but there were most of the solvent extracts have lower stem fresh weight and total fresh weight when comparing to MSO.

Based on the result obtained, we can conclude that the most potential result in promoting hypocotyl and root growth was observed in hexane extract solvent of *Sargassum sp.* as it showed the increment mostly for the all main parameters. According to Hernandez- Herrera *et al.*, (2014), they found that phosphorous in seaweed extracts facilitates root proliferation of *Solanum lycopersicum L.*

However, we found that there were some deficiencies in our study that might affected the seedlings development for both chilli and tomato plants. One of them is the agar plate prepared for chilli and tomato tissue culture had been contaminated. These microbes might give the bad impact to the seeds germination and automatically will affect the seedlings growth.

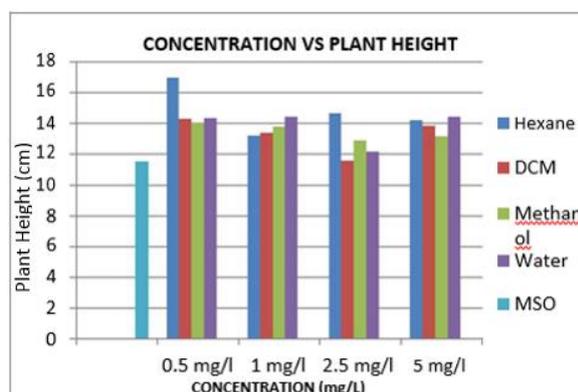


Figure 9. Concentration vs plant height

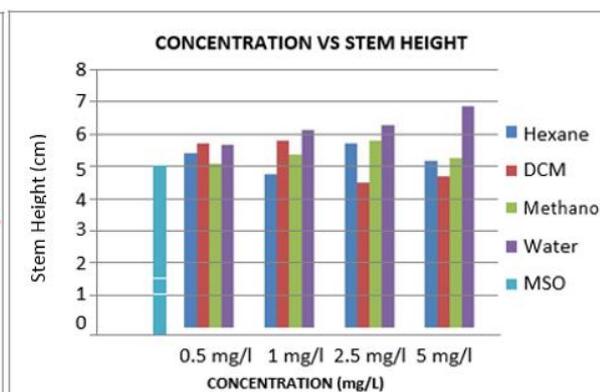


Figure 10. Concentration vs stem height

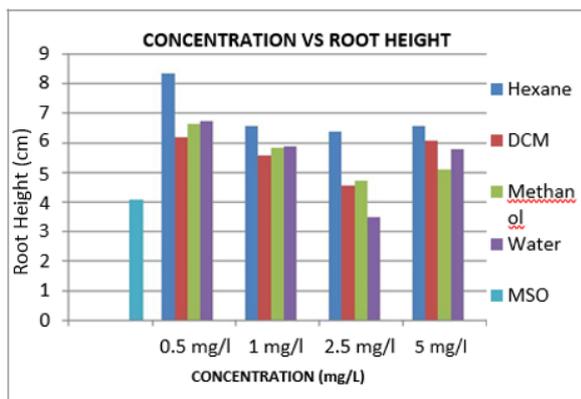


Figure 11. Concentration vs root height

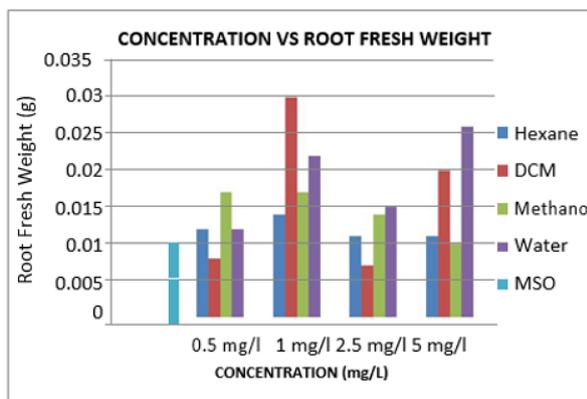


Figure 12. Concentration vs root fresh weight

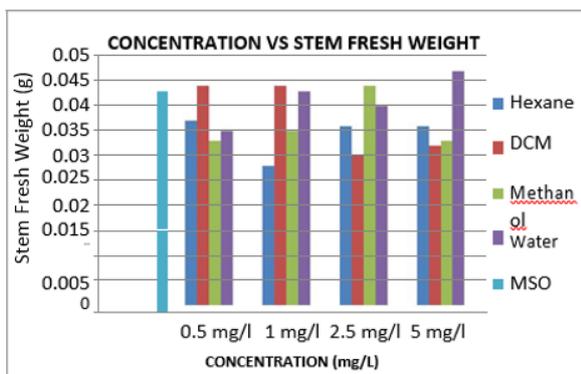


Figure 13. Concentration vs stem fresh weight

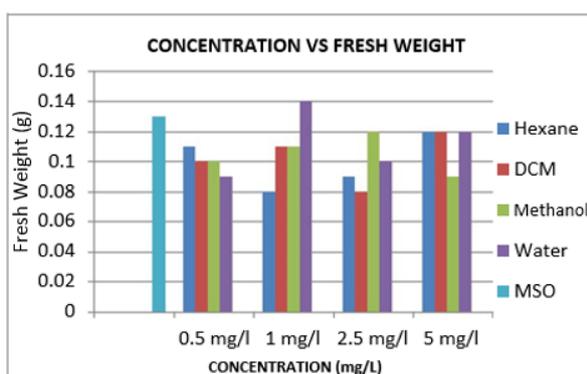


Figure 14. Concentration vs fresh weight

4. CONCLUSION

In conclusion, concentration of 2.50 mg/L can be classified as optimum concentration for seedling development for *Lycopersicon esculentum*. This is because most of the crude solvents showed the positive development in 2.50 mg/L when compared with MSO. However, for *Capsicum annum*, most all of the concentration showed positive increment but we observed that hexane is the most effective extract solvent compared to the others that promote seedling development as most of

the main parameters measurement indicated the increment when comparing with MSO. The effect of *Sargassum* extracts on seedling growth and root development indicates the presence of phtoconstituents that are important to meet the growth requirement.

5. AUTHORS' NOTE

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article. Authors confirmed that the data and the paper are free of plagiarism.

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