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Editor's Note

I am very glad to be an Editor-in-Chief for the Volume 8 of Journal of Dry Zone Agriculture (JDZA) which is published by the Faculty of Agriculture, University of Jaffna, Sri Lanka. The JDZA is set with a keystone of publishing high quality, impactful research findings that address various agricultural and environmental problems with possible scientific solutions. The Volume 8 (Issue 2) of the JDZA, this time, comprises of 5 high quality research papers from various fields: Crop science, Soil science, Economics, Environmental sciences and Food technology. The double-blind review process ensured the high quality of the publications published in this Journal. The JDZA has just been connected successfully to the Sri Lankan Journals Online (SLJOL) platform for the wider display to readers all over the world. Moreover, online submission system of the JDZA is now open throughout the year to accept impactful scientific research papers. I, therefore, welcome authors of scientific fields relevant to the scope of the journal to submit their high quality scholarly works to the JDZA for reaching wider scientific display.

Dr.N.Kannan

Editor-in-Chief/JDZA

Content

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Effect of growing media and irrigation frequencies on early vegetative growth of *Piper longum* L

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Abstract

Systematic cultivation with well-developed agronomic practices for selected medicinal herbs involves conservation of wild populations from over harvesting, continuous supply and sustainable utilization. The present research was conducted to find-out the most suitable growing medium and irrigation frequency for commercial cultivation of *Piper longum* L. For this purpose, six different growing media along with three different irrigation frequencies were used. The experiment was arranged factorially with Completely Randomized Design in triplicates inside a protected plant house under controlled environmental conditions.

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Destructive measurements were taken at 12 weeks after planting. In addition, all growing media were analyzed for pH, electrical conductivity, available nitrogen, organic matter, phosphorous, available potassium using three replicates. Data analysis was carried out using analysis of variance with statistical analysis system while Duncan multiple range test was employed for mean separation. Based on the results, interaction effects between growing media and irrigation frequencies were not significant (P≥0.05) for plant height (cm), number of leaves, number of branches and shoot dry weight (g) of *P. longum* harvested at 12 weeks after establishment. Growing media and irrigation interval were significant on the growth parameters while the highest plant height of 43.3 cm was observed in topsoil: compost -1:1 closely followed by topsoil: sand: compost -1:1:1 (42.2 cm). *P. longum* grown in topsoil: sand: compost -1:1:1 (V/V) had the highest number of branches (7.6). Further, significantly (P≤0.05) higher number of leaves (43) and shoot dry weight (15.7g) were recorded in topsoil: sand: compost -1:1:1. Irrigation frequencies had no significant (P>0.05) effect on growth parameters of *P. longum* (Figure 2). Shoot dry weights had no significant difference with the changing irrigation frequency. Based on the composition analysis and growth parameters, top soil: sand: compost (1:1:1) growing media and 9 days irrigation frequency appeared to be the most promising combination for early vegetative growth of *P. longum.*

Keywords: Composition analysis; Early vegetative growth; Growing media; Irrigation frequency; *Piper longum* L

Introduction

Medicinal plants are natural remedies for wide variety of diseases and disabilities having an exorbitant safety profile. There is a huge demand for medicinal plants in global and local market as they are natural, safe and effective remedies for various kinds of diseases and disorders (Khushbu *et al*., 2011)**.** In Sri Lanka, about 65% of the rural people use ayurvedic medicines as their primary health care (Perera, 2012). In order to cater the steadily increasing demand, most of the medicinal plants are collected from wild resources. Therefore, medicinal plant collectors have imposed great threat on wild resources of medicinal plants due to unsustainable harvesting (Mahindapla, 2004). In addition, Sri Lankan medicinal plant industry is in a risk due to irregular supply of medicinal plants (Perera, 2012), lack of appropriate

cultivation and processing guidelines as well as lack of continuous supply of quality materials (Kankanamalage *et al.,*2013). Sri Lanka owned a variety of soil and climatic conditions and they are favorably influence on growth of many medicinal plants (Kuruppu *et al*. 2019).

Although, a rich genetic base exists for medicinal plants, their propagation and cultivation techniques of very few out of them have been examined (Russell-Smith *et al*., 2006). Thus, extensive efforts are required to recognize their different means of propagation (Walisundara and Watawana, 2014). *P. longum* (family *Piperaceae*) is an imperative medicinal herb with extensive pharmacological characteristics and broad functions. It is one of the essential components in the Ayurvedic medicine (Khushbu *et al*., 2011). It is also known as long pepper. Alkaloids, lignins, piperine, and volatile oils are the major chemical constituents of *P. longum* (Lu *et al*., 2019). Whole plant has the ayurvedic importance (Department of Ayurveda, 2002). Roots contain piperine, piplartine or piperlongumine and mainly dihydro-stigmasterol like important active ingredients (Neelam and Krishnaswamy, 2000). It has been reported wide range of pharmacological properties which includes insecticidal and acaricidal activity, anti-fungal, hepatoprotective, anti-asthmatic, anti-microbial, diabetic, inflammatory, anti-cancer, antioxidant, analgesic and depressive properties (Zaveri *et al*., 2010).

There are no large-scale organized cultivations of *P. longum* in Sri Lanka. In Sri Lanka *P. longum* is one among the 10 largely imported herbal materials due to its increasing demand for the ayurvedic preparations (Gunathilake *et al,* 2002). Therefore, it is important to establish systematic commercial cultivations of *P. longum* to ensure continuous supply of raw materials to cater the steadily increasing demand. Current literature lacks information about the correct combinations of irrigation and proper potting media required for the *P. longum*. In addition, it is beneficial to develop medicinal plant cultivations with the use of organic growing substrates. Importantly, medicinal plants cultivated without synthetic chemical fertilizers possess high quality, efficacy and reliability of herbal materials and can extract authentic pure form of secondary metabolites (Kankanamalage *et al*, 2013). Hence, present study was designed to find-out appropriate organic growing medium along with suitable irrigation frequency for better growth and development of *P. longum* at early vegetative growth.

Materials and methods

The present study was carried out inside a plant house under controlled environmental conditions located at Faculty of Agriculture, University of Ruhuna during December 2020 - March 2021. During the study period, average monthly temperature, RH and the light intensity of the protected plant house were 33^0C, 70% and 25000 lux respectively. Six different growing media [T_1 - Topsoil: sand: compost (1:1:1); T_2 - Topsoil: sand: compost (1:1:2); T_3 - Topsoil: compost (1:1), T_4 - Topsoil: compost (1:2); T_5 - Topsoil only and T_6 - Topsoil: sand (1:1)] along with three irrigation frequencies (3, 6 and 9 days) were used as eighteen different treatments. They were factorially arranged in Completely Randomized Design with three replicates [MI_1, MI_2 and MI_3- growing media 1 with 3, 6 and 9 days irrigation frequency intervals; MI_4, MI_5, MI_6- growing media 2 with 3, 6 and 9 days irrigation intervals; MI_7, MI_8, MI_9 - growing media 3 with 3, 6 and 9 days irrigation intervals; MI_{10}, MI_{11}, MI_{12} -growing media 4 with 3, 6 and 9 days irrigation frequency; MI_{13}, MI_{14}, MI_{15}- growing media 5 with 3, 6 and 9 days irrigation frequency; T_{16}, T_{17}, T_{18}- growing media 6 with 3, 6 and 9 days irrigation interval].

Six weeks old rooted plantlets of *P. longum* were planted in black polythene pots of 10"×10". These pots were filled with six different growing media as mentioned above. At each water application, about 250 mL water was applied to reach the field capacity determined by gravimetric method. Data on plant growth parameters such as plant height (cm), number of leaves, number of branches, shoot dry weight (g), root dry weight (g) and root volume (mL) were collected at 12 weeks after establishment. Physio-chemical parameters of six different growing media including pH, Electrical Conductivity (EC), organic matter content, available phosphorus content (mg/kg), potassium (mg/kg), ammonium nitrogen (mg/kg) and nitrate nitrogen (mg/kg) were collected. Data on pH and EC were determined using the pH and EC meter (HANNA HI 83099). Ammonium nitrogen, nitrate nitrogen and available phosporous were analysed using UV visible spectrophotometer (Shimadzu UV160). Available potassium was measured using the flame photometer (Sherwood model 360).

Data were subjected to analysis of variance with the help of statistical analysis system (SAS statistical software 9.1 version). Duncan multiple range test was employed to compare treatment means. A Pearson correlation (SPSS software) was carried out to test the relationship/s among chemical composition and early vegetative growth of *P. longum* grown in different growing media.

Results and discussion

Interaction effects between growing media and irrigation frequencies were not significant ($P \geq 0.05$) for plant height (cm), leaves number, branches number and shoot dry weight (g) of *P. longum* harvested at 12 weeks after establishment. Therefore, results of main effects are presented for these parameters. However, significant ($P \leq 0.05$) interactions between growing media and irrigation frequencies were observed for dry weight of roots and volume of roots (mL) at the same stage.

Effect of different growing media on growth and yield of *Piper longum* L

Different growing media differently influenced on plant height (cm), number of leaves, number of branches and shoot dry weight (g) of *P. longum* harvested at 12 weeks after establishment. The highest plant height of 43.3 cm was observed in T_3 (topsoil: compost - 1:1) closely followed by T_1 (topsoil: sand: compost - 1:1:1) (Figure 1A). *P. longum* grown in T_1 (topsoil: sand: compost - 1:1:1) had the highest number of branches (7.6) (Figure 1C). Significantly ($P \leq 0.05$) higher number of leaves (43) and shoot dry weights (15.7g) were recorded in T_1 (topsoil: sand: compost - 1:1:1) (Figure 1B and D). Topsoil: compost (1:2) growing media showed the lowest values for plant height (31.7cm), leaves number (13), branches number (4) and shoot dry weight (4g) (Figure 1).

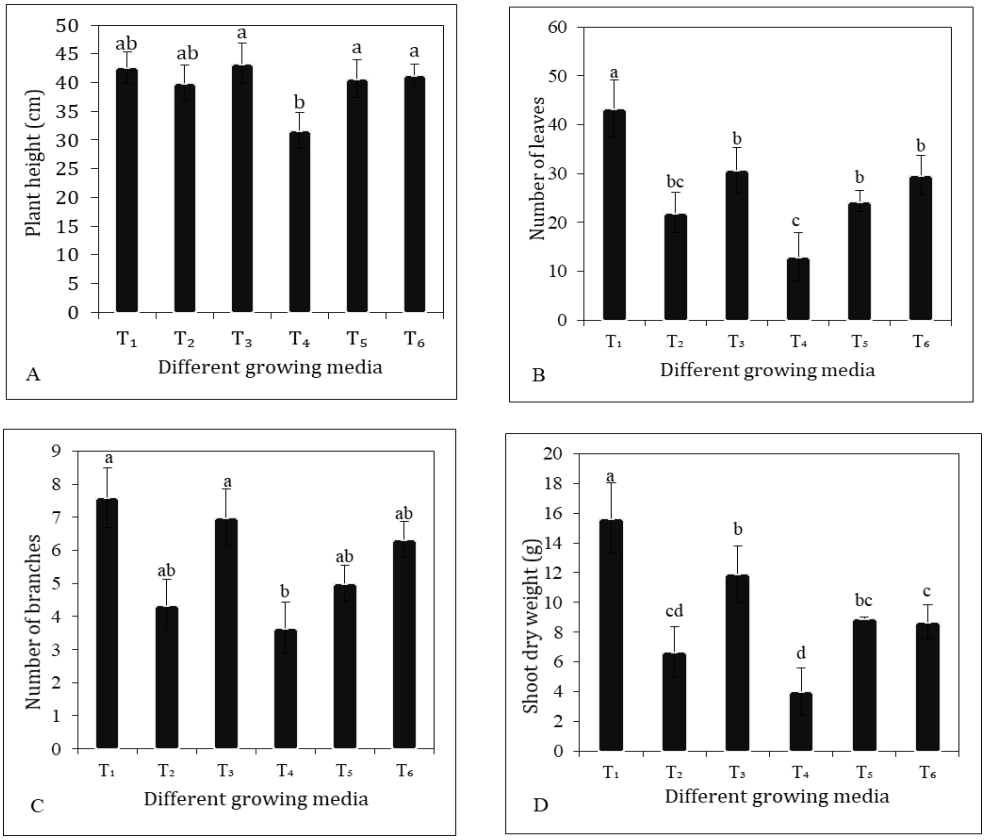

Figure 1: Influence of different growing media on plant height (cm)(A), number of leaves (B), number of branches (C), and shoot dry weight (g)(D) of *P. longum* grown under protected house conditions [T_1 - topsoil: sand: compost - 1:1:1, T_2 - topsoil: sand: compost - 1:1:2, T_3 - topsoil: compost - 1:1, T_4 - topsoil: compost - 1:2, T_5 - topsoil only and T_6 - topsoil: sand - 1:1].

Results of the present study report that topsoil: sand: compost - 1:1:1 and topsoil: compost - 1:1 as the most appropriate growing media for early vegetative growth performances of *P. longum*. Based on the literature, topsoil: sand: compost 1:1:1 was a recommended medium for different crop species including clove bean (Munasinghe and Fernando, 2021). Similarly, Weerasingha (2020) had used growing media consist of topsoil: sand: compost 1:1:1 as the standard growing media for *Capsicum annum*. Moreover, potting mixture containing topsoil: sand: compost: coir dust (1:1:1:1) and top soil: sand: compost: coir dust (1:1:1:1) showed the highest growth performances in betel belongs to same family of *P. longum*. The lowest values for all the above

parameters (Figure 01) were reported in T_4 (topsoil: compost 1:2) although, it consists of higher fraction of compost.

Observed growth retardation in the topsoil: compost 1:2 may be due to unfavorable effects from high amount of compost. The nutrient composition such as nitrate nitrogen, ammonium nitrogen, available phosphorus content, potassium and organic matter content of the compost used in the present study were respectively, 4.8 mg/kg, 0.36 mg/kg, 178.2 mg/kg, 908 mg/kg and 16%. Nitrogen mineralization rate was the main factor determining the growth and development of the plants and it appears that the use of compost at the correct proportion with other optimum nutrient levels can claim for a higher growth and yield (Raviv *et al*., 2004). Hose *et al*., (2012) also emphasized that growth characteristics and quality of crop yield were differed according to the components used to prepare compost and their proportions. Top soil: compost 1:2 (T_4) had significantly lower growth rate possibly due to poor drainage caused by the absence of sand and presence of high amount of compost. Growing media compaction was observed as fewer voids with lack of sand in the medium. Topsoil with compost help to retain water and provide nutrients and sand increase drainage and improve aeration. Plant may get optimum amount of moisture, aeration, nutrients and improved drainage as growing media 1 (T_1) contains equal amount of topsoil, sand and compost.

Effect of different irrigation frequencies on growth and development of *Piper longum* L.

Irrigation frequencies had no significant ($P>0.05$) effect on growth parameters of *P. longum* (Figure 2). There was no significant difference ($P>0.05$) in shoot dry weights with the changing irrigation frequency. Rao *et al*., 2010 found that a significantly higher response in growth, yield and quality of *Piper longum* was with irrigation regime of 0.8 cumulative pan evaporation and emphasized that *P.longum* required comparatively high watering. Joshi *et al*., 2013 emphasized that *P. longum* need to irrigate at least once a week if it grown as a sole crop. Irrigation and water content of the growing media has an influence on plant growth and development as well as runoff process. A potted plant must be able to equilibrate the atmospheric demand for water with the amount it absorbs from the growing media. Plants can withstand only for the range of readily available water in the growing media up to the refill point to the

container capacity (Wang 2013). Nowadays, irrigation frequency and growing media water content has an increasing concern as it transports chemicals and potentially can harm surface or groundwater (Smith, 2007).

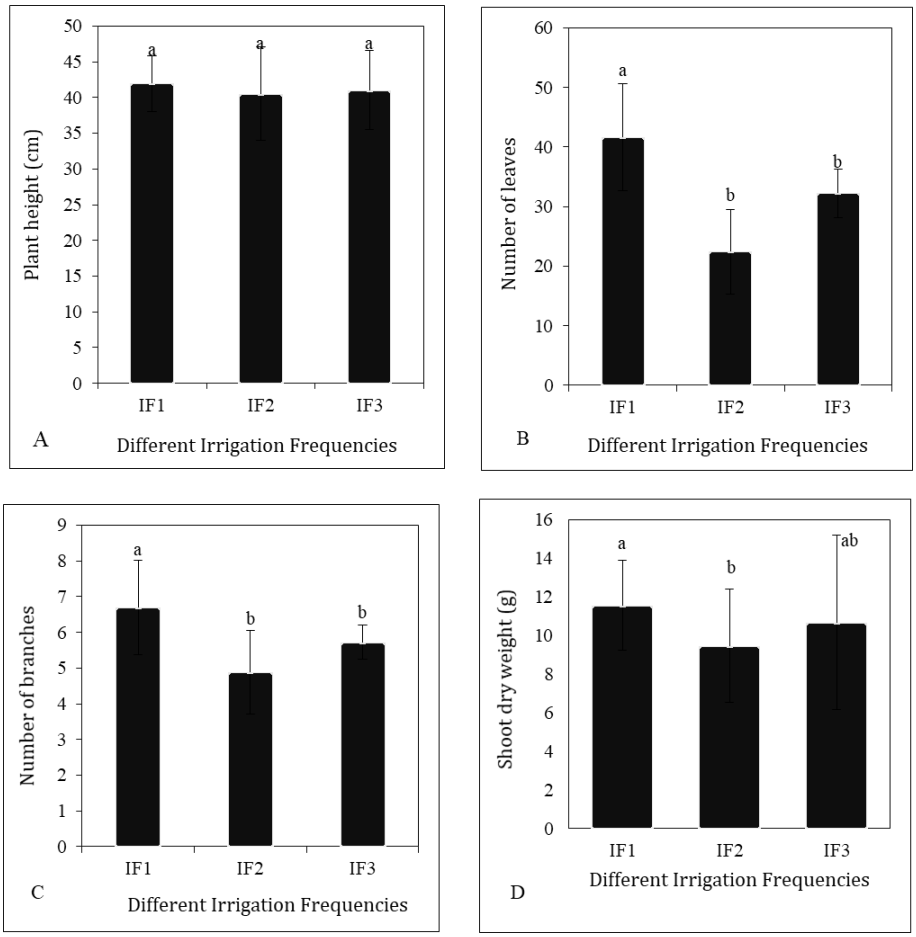

Figure 2: Influence of different irrigation frequencies on plant height (cm)(A), number of leaves (B), number of branches (C), and shoot dry weight (g) (D) of *P. longum* grown under protected house conditions [IF1, IF2 and IF3 - 3, 6 and 9 days irrigation frequencies respectively]

Effect of growing media and irrigation frequencies on root parameters

Root parameters had an interaction effect with growing media and irrigation frequency. Significantly ($p<0.05$) highest root dry weight (4.6g) and the highest root volume (22.6 ml) were recorded in top soil: sand: compost 1:1:1 when irrigated at 9 days interval (MI_3) (Table 1).

Table 1: Interaction effect of growing media and irrigation frequency on root dry weight (g) and root volume (mL) of *P. longum* harvested at 12thweeks after establishment

Treatment	Root dry weight (g)	Root volume (mL)	Treatment	Root dry weight (g)	Root volume (mL)
T_1	3.3^{b}	13.3^{bc}	T_{10}	1.2def	7cdef
T_2	2.8^{bc}	19.6^{a}	T_{11}	0.5^{f}	2.6^{f}
T_3	4.6^{a}	22.6^{a}	T_{12}	0.8^{ef}	5.3^{ef}
T_4	1.4def	5.5^{ef}	T_{13}	1.8cde	12.3^{bcd}
T_5	1.2def	5f	T_{14}	2.2^{cd}	11.6bcde
T_6	1.9^{cd}	8.3bcdef	T_{15}	2cd	13.6^{b}
T_7	2.1^{cd}	7.6bcdef	T_{16}	1.3def	6def
T_8	2.8^{bc}	12.6^{bc}	T_{17}	1.6^{de}	8.3bcdef
T_9	1.2def	5.6^{ef}	T_{18}	1.8cde	8bcdef

Note: Means with similar letters in a column are not significantly different at $p<0.05$

Composition analysis of different growing media

Composition analysis results indicated that (Table 2), different growth media showed significant differences ($P<0.05$) for pH, electrical conductivity (dS/m), nitrate nitrogen (mg/kg), ammonium nitrogen (mg/kg), available phosphorus (mg/kg), available potassium (mg/kg) and organic matter content (%). Significantly higher pH was observed in T_1, T_2, T_3 and T_4. Potting media with topsoil: compost 1:2 (T_4) showed the highest nitrate nitrogen content. Ammonium nitrogen and phosphorus were greater in T_3 (Table 2). Various chemical and biological parameters such as pH, EC, total organic carbon content (TOC), nitrogen content, carbon nitrogen (C:N) ratio, humic like substances,

enzymatic activities, ATP content considered as indicator of compost stability (Mondini, 2004). Compost phytotoxicity is an important criterion as the application of immature compost will have higher amount of free ammonia, organic acids and other water-soluble compounds, which can cause plant growth retardation and root development (US Composting Council, 2002). These reasons may be associated with the growth retardation observed in media with high proportions of compost.

Table 2: Composition analysis of different growing media

Treatments	pH (w/v)	EC (dS/m)	Nitrate Nitrogen (mg/kg)	Ammonium Nitrogen (mg/kg)	Phosphorus (mg/kg)	Potassium (mg/kg)	Organic Mater (%)
T_1	7.8^{ab}	1.21^{d}	1.6^{e}	0.76^{c}	126.5^{d}	193.0^{e}	4.6^{f}
T_2	7.9^{a}	2.03^{c}	2.7^{d}	0.79^{c}	139.6^{c}	408.0^{c}	6.4^{e}
T_3	7.7^{ab}	2.80^{b}	4.0^{a}	1.37^{a}	182.7^{b}	568.9^{a}	15.1^{a}
T_4	7.7^{ab}	3.45^{a}	5.1^{a}	1.24^{b}	156.5^{a}	815.0^{a}	13.4^{b}
T_5	6.6^{c}	0.11^{e}	3.3^{c}	0.76^{c}	13.4^{e}	3.6^{d}	7.9^{c}
T_6	7.4^{b}	0.04^{e}	3.2^{f}	0.42^{d}	10.7^{f}	0.3^{f}	2.3^{d}
CV%	3.08	8.73	2.33	0.3	1.53	0.2	0.5
p	0.006	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

[T_1 - Topsoil: sand: compost 1:1:1, T_2 - Topsoil: sand: compost 1:1:2, T_3 - Top soil: compost 1:1, T_4 - Topsoil: compost 1:2, T_5 - topsoil, T_6 - Topsoil: sand 1:1]

Note: means with similar letters in a column are not significantly different at $p<0.05$

Correlation coefficients were used to estimate the strength and direction of the relationship between the plant growth parameters and physio-chemical characters of different growing media (Table 3). Direction and the strength of association was nutrient dependent. For example, Nitrate N in growing media and K were negatively correlated with plant growth parameters. Further,

negative correlation of Nitrate N and K showed significant ($P \leq 0.01$) influence on shoot dry weight (g), number of branches and number of leaves (Table 3). Thus, it is important to select a growing medium with relatively low levels of Nitrate N and K in order to achieve the higher growth of *P. longum.* Increased application of P and organic matter had the positive relationship with improved growth. The availability of P in the potting media has a direct impact on nitrate metabolism (Linkohr *et al*., 2002). One of the main reasons of nitrate accumulation in most plants is an uneven and greater supply of nitrogen and P (Perring *et al*., 2018). Nitrate N is a significant form of nitrogen absorbed by plants, and Ammonium-N fertilizer can be converted to Nitrate-N relatively quickly when applied to aerobic soil, increasing the soil Nitrate-N content (Schroeder and Janos, 2005).

Table 3: Relationship between growth parameters of *P.longum* and physic-chemical characteristics of different growing media

	pH	**EC**	**OM**	**Am N**	**Nitrate N**	**P**	**K**
Root dry weight	0.24	0.09	0.16	-0.12	-0.12	0.16	-0.12
Root volume	0.27	0.11	0.14	-0.11	-0.02	0.14	-0.07
Shoot dry weight	0.4*	0.09	0.02	-0.03	-0.54**	0.02	-0.4*
Number of Branches	0.27	0.04	0.01	-0.07	-0.52**	0.01	-0.48**
Number of Leaves	0.34	0.13	0.11	0.07	-0.57**	0.1	-0.48**
Plant height	0.03	0.01	0.11	0.01	-0.24	0.11	-0.19

(Am N - Ammonium nitrogen, P - Available Phosporus, K - Available Potassium)

** Significant level at $P \leq 0.01$ and * Significant level at $P \leq 0.05$

Conclusion

Topsoil: sand: compost (1:1:1) media and 9 days irrigation frequency appeared to be the most favorable growing media and irrigation frequency for *P. longum* early vegetative growth. Further studies are essential to evaluate influence of

growing media and irrigation frequencies on spike yield of *P. longum.* In addition, optimum compost level and toxic conditions with excess application of compost for *P. longum* warrant further investigations.

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A preliminary study of cost and energy analysis of bio-fuel production from microalgae cultivated in parboiled rice mill wastewater

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Abstract

Microalgae industry is rapidly growing industry with high potential for value added products. Especially microalgae derived biofuels provide a sustainable solution for continuously rising energy crisis, food security and greenhouse gas emission. However, at present economical limitations make this green fuel not commercially viable. Integrating wastewater treatment with the microalgae cultivation can significantly reduce the biofuel production cost. Parboiled rice mill is rich in nutrients and capable of providing inexpensive water resources. Nevertheless, energy and cost analysis are required in order to ensure the cost effectiveness of such approaches. Thus, the study evaluates the cultivation of microalgae in rice mill wastewater in the outdoor uncontrolled conditions for the cost and energy effective, biofuel production. Microalgae biomass was cultivated in low-cost poly-bags of 3L volume using parboiled rice mill wastewater in outdoor. Cultivation was done under ambient temperature with 0.2vvm aeration without any optimization of the natural environmental

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conditions. This can be rationalized to reflect a realistic scenario in terms cost and energy estimation of production of lipid and bio-ethanol at laboratory scale. The obtained bioethanol and lipid yield were 1.4% and 5.81% respectively. Cultivation of the microalgae consumed 2.16 MJ of energy per gram. The required energy content for production of bioethanol and lipid from a gram of biomass were 3.61764 MJ and 3.5336 MJ respectively. Cost of microalgae cultivation to produce gram of bioethanol and lipid were $ 22.91 and $ 5.52 respectively. Total production cost per gram of bioethanol and lipid were $ 64.73 and $ 55.91 respectively. These values are comparatively exorbitant. Since the production was at very small scale and the yield was very low due to the microalgae cultivation in outdoor under natural condition in laboratory scale. The production of biofuel could become economically feasible if the yield could optimally increase and simultaneous production of bioethanol and lipid along with other value added products such as biofertilizer, aquaculture feed etc. could be achieved. Therefore, the method resulted in the lower cultivation cost can be incorporated with the residual biomass utilization and could produce sustainable microalgae biofuel.

Keywords: Microalgae; Rice mill wastewater; Lipid; Bioethanol; Energy; Cost analysis

Introduction

Microalgae production is a rising industry with very high potential. Even during COVID -19 pandemic in 2020 the global market was estimated as 3.4 billion USD and projected to reach 4.6 billion USD by the year of 2027(Show, 2022). Microalgae contain range of valuable bioactive compounds including: lipids; proteins; carbohydrates; carotenoids and vitamins (Tan *et al.*, 2020). Thus microalgae biomass has great value as bio-plastics, bio-fertilizer, pharmaceutical and cosmetic active ingredients, food source, aquaculture and animal feed, CO_2 capture, wastewater treatment system and biofuels (Daneshvar *et al*., 2019; Sakarika *et al*., 2020). Especially microalgae derived biofuels provide a sustainable solution for continuously rising energy crisis, Greenhouse Gas (GHG) emissions, and food security issues due to using food crops for biofuel production. Hence the biofuel from microalgae is a renewable and sustainable alternative energy source to secure the future energy demand (Pokoo-Aikins *et al*., 2010; Zewdie and Ali, 2022).

Microalgae typically have high growth and productivity, ability to grow on wastewater, saline or seawater and potential for high lipid or carbohydrate contents which make it an excellent feed stock for range of biofuels (Branco-Vieira *et al*., 2020). Algal lipids are composed of glycerol, sugars or bases which are trans esterified to saturated or unsaturated fatty acids to produce biodiesel (Halim *et al*., 2012). Carbohydrates is another major component which can be readily used as feedstock to produce bioethanol. Since they are characterized by high concentration of easily fermentable cellulose and starch, along with low concentration of hemicelluloses and absence of lignin (Sakarika *et al*., 2020; Tan *et al*., 2020). The whole biomass can be subject to anaerobic digestion to produce biogas. Further it can be done using residual biomass after the lipid extraction as well. Finally, the production of bio hydrogen is a clean energy through direct biophotolysis or dark fermentation (Sakarika *et al.*, 2020). If more than one biofuel or another value-added product with biofuel could be produced with wastewater treatment, then the whole approach will be highly sustainable, economically feasible and environmentally friendly (Rafa *et al*., 2021).

However, at present commercial facilities producing microalgae are limited to high-value applications such as food, cosmetics, and pharmaceuticals active components etc. Because, the current high microalgae production cost cannot be afforded by other low value market products such as biofuel (Acién *et al*., 2016). The cost for microalgal biomass production is currently much higher than the cost for production of other energy crops (Bušić *et al*, 2018; Zewdie and Ali, 2022). Therefore, in the recent decade numerous studies have explored various technologies and implementation techniques to make the microalgae biofuel production cost effective enough to be competitive to traditional fuels in the global market (Chen *et al*., 2018). Among them, integration of microalgal cultivation for biofuel production with wastewater (WW) treatment for both low cost nutrient and environmental pollution reduction was one of the promising process that resulted in significant reduction in the production cost (Zewdie and Ali, 2022). The ALL-GAS project, led by FCC Aqualia is an excellent example of this. The project demonstrates the sustainable large-scale production of biofuels based on low-cost microalgae cultures using municipal wastewater (https://www.all-gas.eu/).Wastewater is rich in growth nutrients like nitrogen, carbon and phosphorous and trace elements that are essential for

micro algae growth, which consequently result in the phytoremediation of the wastewater. In addition, the ability of microalgae to remove, organic and inorganic pollutants including heavy metals and emerging contaminants is well documented in the literature (Kanaujiya *et al*., 2019; Li *et al*., 2022; Sakarika *et al*., 2020).

Parboiled rice mills are one of the important and oldest industry in the developing countries like Sri Lanka where rice is the staple food crop. Worldwide rice milling production is approaching about 500 million metric tons in 2021 (Mukherjee *et al*., 2016). They play key role in both the national and rural economy and of the food security. However, they produce huge amount of wastewater which is rich in nutrients and typically have very high chemical oxygen demand is reported to be discarded without any remediation treatment into environment. This results in significant eutrophication, surface and ground water pollution, and wastage of large quantities of utilizable water (Umamaheswari *et al*., 2021). Integrating this wastewater treatment with microalgae production system could provide a win-win situation. In our previous study microalgae, recovered 88% of total nitrogen and 75% of phosphate. Further the chemical oxygen demand was reduced by 87%, emphasizing the enhancement of the parboiled rice mill wastewater (Ketheesan *et al*., 2021).

However, experimental validations are still required for converting wastewater cultivated microalgae biomass into value added products such as biofuel. Energy and cost analysis should have been carried out in order to determine the feasibility of the system (Judd *et al*., 2017). This is one of the most significant issues for any industrial application of research output as economic feasibility is the major concern of commercial execution of any product. There are several life cycle assessment studies, Techno economic analysis and energy balance studies have been performed to investigate the commercial scale, real world application of biodiesel production from microalgal biomass (Branco-Vieira *et al*., 2020; Dutta *et al*., 2016; Hossain *et al.*, 2019). These studies have certain limitations, that is, typically they consider very optimal conditions for microalgae growth and higher lipid, carbohydrate and biomass productivity for microalgae. Moreover, the studies include theoretical assumptions, and often lack original data (Hossain *et al.*, 2019; Show, 2022).

Thus the present study is a novel approach to produce microalgae biomass by using inexpensive nutrient and water sources obtained from rice mill factory in outdoor natural conditions without any control measurement. Through this, the study investigates more realistic answers in terms of cost and energy estimation for the production of lipid and bio ethanol at laboratory scale. Further, the study analyses whether the cost reduction by the utilization of wastewater and lack of control measurement could provide enhanced cost and energy efficiency.

Materials and method

Microalgae cultivation

Chlorella sp. which was isolated from polluted lakes in Jaffna district and grown in the Environmental laboratory of the Civil Engineering department, Faculty of Engineering, University of Jaffna, Killinochchi was selected and obtained for the research purpose. Stock culture was grown inside the laboratory in bold basal medium under ambient temperature. Culture was maintained under light intensity of 500 PAR (16h: 8h) and mixing of 18 RPM.

Collection of rice mill wastewater

Parboiled rice mill wastewater was collected from the rice mill situated in Killinochchi town area. As a pretreatment, sedimentation of rice mill wastewater was done. The characterization of the wastewater, recovery of nutrients and treatment efficiency of the rice mill wastewater by microalgae was done. The rice mill wastewater was characterized by very high COD (>2500mg/L), Phosphate (471 mg/L), Sulphate (650 mg/L) and Nitrate (340 mg/L) and at average microalgae recovering more than 70% of nutrients. The full results were published in the previous work (Ketheesan *et al*., 2021).

Cultivation and harvesting of microalgae in rice mill wastewater

Microalgae were cultivated outdoors in about six 3-L high gauge polythene bags (Polybags). Microalgae were inoculated with an initial biomass concentration of approximately 20-30 mg/L and grown for 7 days. No additional lighting and CO_2 supply was provided. Aeration at 0.2 vvm was provided to ensure the mixing of the culture. At the end of cultivation period biomass was allowed to

settle and effluent was removed. Biomass was harvested by centrifugation, dried at 70 °C and ground to fine particles.

Valorization of microalgae biomass

Bioethanol

Five gram of microalgae biomass was dissolved in 100 mL distilled water and sonicated. The obtained supernatant was fermented to ethanol by *Saccharomyces cerevisiae.* It was cultured in LB medium. The dichromate oxidization method was used to determine the amount of ethanol extracted. The extraction was done in triplicates.

Lipids

One gram of biomass was added to hexane/methanol 7:3 (v/v) and sonicated. Extraction of lipids was done by Soxhlet extraction method (Buchi B-811 system). Extraction was carried out for 2 hours followed by 10 minutes of rinsing and 5 minutes of drying in the rotatory evaporator. The extraction was done in triplicates.

Cost analysis

Economic evaluation typically consists of capital cost, variable operation costs and operational cost (Zewdie and Ali, 2022). The study didn't include capital cost and operational cost as this is a laboratory scale experiment and established systems were used. Therefore, only the variable operation cost that includes power and raw materials was considered. Cultivation of microalgae biomass, Harvesting, Bioethanol production, Extraction of lipids were identified as a major stage for the cost analysis.

Results and discussion

Bioethanol and lipid yield

Microalgae ethanol and lipid yield were 1.4 ± 0.04% and 5.81 ± 0.69% respectively. These values are in the very lower range compared to similar studies. Typically, 12%-32% of lipid content and 4-20% ethanol content were recorded in the literature (Bušić r *et al*., 2018; Hossain *et al*., 2019; Li *et al*., 2022). Uncharacteristically low value recorded in the study may be due to the

fact that the cultivation was done in outdoor under natural conditions in very small scale. Higher yield was often obtained in indoor cultivation and large scale outdoor cultivation where the impact of environment conditions can be diluted or diffused (Branco-Vieira *et al*., 2020).

Further, microalgae have been known to survive under a wide range of conditions. Compared to unfavorable conditions, favorable conditions result in lower storages of lipid and carbohydrate. Thus, various studies emphasized that nutrient starvation, especially phosphorus limitation, significantly enhance the carbohydrate and lipid content of the biomass (Hanifzadeh *et al.,* 2018, Rehman and Anal 2019, Shrestha *et al.*, 2020). Hence, it is ideal to cultivate microalgae under optimal conditions and later expose them to unfavorable conditions such as nutrient starvation in order to increase lipid and carbohydrate content (Pokoo-Aikins *et al*., 2010).

Energy and cost analysis

Energy required for the production of bioethanol and lipid were 3.62 MJ and 3.53 MJ respectively. The value was obtained for the production and processing of gram of microalgae in a single cycle. Energy requirement for the mixing was the highest (Table 1) in this study. In order to prevent the sedimentation, mixing was employed here. To small scale production such as this manual mixing could reduce this cost. However, in large scale production such as raceways and photo bioreactors as well as mixing utilize significant energy that impact the production cost.

Table 1: Energy consumption at various stages of the process.

Production stages	**Process**	**Equipment**	**Energy used for required capacity**	
			kWh	**MJ**
Microalgae Cultivation	Mixing	Air pump	0.600	2.16
	Harvesting	Refrigerated centrifuge	0.12375	0.4455

Table 1: Energy consumption at various stages of the process. (Continued)

Raw material preparation (microalgae)	Drying	Incubator (BI-BSP-100)	0.02400	0.08640
Bioethanol production	Cell destruction	SONIC water bath	0.00047	0.00169
	LB medium preparation	SN 30 sterilizer	0.00330	0.01188
	Storing	Bio Base refrigerator	0.00005	0.00018
	Yeast culture	Incubator (BI-BSP-100)	0.00013	0.00047
	Yeast culture mixing	Shaking Incubator (BSD-250)	0.03720	0.13392
Lipid extraction	Cell destruction	SONIC water bath	0.00022	0.0008
	Extraction	Buchi B-811 Soxhlet	0.01758	0.0633

The second highest energy intensive process was harvesting. This is especially true for large scale production as centrifugation is very energy intensive (Bušić *et al.*, 2018). This account for about 30% of their expenditure ($2.92–3.06/L biodiesel) (Chen *et al*., 2018; Rafa *et al*., 2021). However, the biomass cultivated in the rice mill wastewater showed high settle ability. Thus, tropical countries like Sri Lanka can employ solar drying for the cost-effective harvesting of the biomass (Pokoo-Aikins *et al*., 2010).

Chemical cost is the highest for processing 1g microalgae for both bioethanol and lipid production (Table 2).

Table 2: Cost of cultivation and processing of a gram of microalgae

Production stages	**Supplies**	**Total cost**	
		Rs.	**$**
Cultivation	Polyethylene bags	5.00	0.014
	aeration tubes and pump (NS L-15)	110	0.3056
	Energy cost	8.83	0.025
Harvesting	Energy cost	2.17	0.006
Bioethanol production	chemicals	210.275	0.584
	Energy cost	0.61	0.0017
Extraction of Lipids	Chemicals	205.92	0.572
	Energy cost	0.26	0.00073

In terms of total production, cost of lipid was lower than the bioethanol since the lipid yield was higher (Table 3).

Table 3: Production cost of bioethanol and lipid

	For 1g of bioethanol	**For 1g of Lipid**
Required Microalgae biomass	71.43 g	17.21 g
Microalgae cultivation cost	$22.91	$5.52

Table 3: Production cost of bioethanol and lipid (Continued)

production cost	$ 41.82	$ 50.39
Total cost (Cultivation cost + Production cost)	$ 64.73	$ 55.91

The cultivation cost is lower in the studies. This may be attributed to the integration of the process to wastewater treatment. To cultivate 1g of microalgae biomass $ 0.34 is required in the present study. If we extrapolate this to produce 1kg of the biomass, it will be about $ 6.42 since the energy and raw material cost remains fixed up to certain amount of microalgae biomass. This value is comparable to other studies which reported production cost of microalgae biomass grown in wastewater between $ 2 to $ 15 (Branco-Vieira *et al*., 2020; Rafa *et al*., 2021).

Energy and cost values for the production and processing of 1g microalgae is comparable to the other studies. However, the production cost of bioethanol and lipid is very high in the present study. Similar studies reported production cost of $ 2.8 to 6.7 per liter of biofuel (Judd *et al*., 2017). In the study by Branco-Vieira *et al*.(2020) production cost of biodiesel was estimated as $ 0.33/L and of biomass $ 0.0022 per g, in a 15.247 ha facility size. The high production cost in the present study is due to very low yield obtained in the study. Low yield results in the requirement of large amount of microalgae biomass and consequently the production cost is increased several fold for the processing of this large amount of biomass (Table 3).

Average price of liter of ethanol is $ 1.16 that means one gram of ethanol is $ 0.0015 in 2022. Current price of algae biomass is $ 19000 per ton means $ 0.021 per gram (Show, 2022). Biodiesel price is $ 5.34 per gallon (United States, Department of Energy). Biofuel from microalgae to be economically feasible, even the lowest price reported in the literature necessitates to substantially reduce their cost and to operate them near their optimum values.

Therefore, in addition to integrating wastewater treatment, simultaneous production of different biofuels along with other value added products such as: biofertilizer; aquaculture feeds; proteins; enzymes etc. It I s recommended by several authors (Bielsa *et al*., 2016; Dutta *et al*., 2016; Rafa *et al*., 2021). The

study by Gupta *et al*., (2016) reported that when the protein is also extracted, cost of biodiesel production could be reduced from $17.26/L to $13.73 US/L. In another study by Prieto *et al*. (2017), production cost of biodiesel from microalgae was found to reduce from $ 3.90/L to a staggering $ 0.54/L when astaxanthin and polyhydroxy butyrate are coproduced. Further, Chen *et al*. (2018) reported that despite the importance of the lipid content of the microalgae, often it does not significantly affect the parameter of cost estimation if the microalgae residues are assigned an economic value (Rafa *et al*., 2021).

Regardless of these, there are numerous hurdles that need to be handled related to the cost of current technologies for the widespread commercialization of microalgae-based biofuel. The present study shows that even at laboratory scale with integrating wastewater, the production cost is extremely high. To bring microalgae biofuel production to be competitive in the global market alternate technologies and novel approaches are required. However, still microalgae are having very high potential in the biofuel field. Dwindling fossil fuel reserve and controversy over utilization of food crops for biofuel when the food security is at stake with ever increasing population makes the microalgae an inevitable alternate source.

Conclusion

Bioethanol and lipid yield obtained from microalgae biomass was 1.4% and 5.81 % respectively. This uncharacteristic in this study may due to the fact that the cultivation was done in outdoor under natural conditions in very small scale. Comparatively lower cultivation cost was achieved in the study ($ 6.42 per Kg) as no lighting systems and additional CO_2 supply were provided and cultivation was carried out in wastewater medium using low cost poly bags. However, due to the low yield, production cost of bioethanol and lipid increased several folds compared to other studies. The present study shows that even at laboratory scale with integrating wastewater, the production cost is extremely high. However, since the cultivation cost is lower if the residual biomass could be utilized to produce value added products such as proteins, biofertizer, aquaculture feed could make the process more cost effective. Further simultaneous production of various biofuel such as bioethanol, biodiesel, biogas and bio hydrogen can also make the microalgae derived biofuel production

more sustainable, energy and cost effective and feasible in the commercial scale applications. Therefore, the method resulted in the lower cultivation cost can be incorporated with the simultaneous production of various value added products and could produce sustainable microalgae biofuel.

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Development of spray drying technique for papaya (*Carica papaya*) fruit juice powder

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Abstract

Post-harvest losses of *Carica papaya* are considerably high throughout the supply chain due to variety of reasons. Spray drying is one of the potential preservation technologies to address this issue. Therefore, this research was conducted to identify the most acceptable carrier agent for papaya spray drying. The final quality of the product was tested with Maltodextrin and Glucose syrup with three different concentrations and at three different temperatures. Processing temperatures of 60 °C, 80 °C, and 100 °C were employed, with three concentrations (7.4%, 11.5%, and 15.3%) of carrier agents. The results revealed that temperature and carrier agent concentration had no effect on ascorbic acid, solubility, and pH (P>0.05). But the titratable acidity, moisture content and rehydration duration of dried powder differed. With increasing temperature, ascorbic acid and moisture content decreased while the pH of the solution increased with temperature and concentration.

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Changes in temperature have significantly effect on rehydration time. Better rehydration times were observed for maltodextrin and glucose syrup concentrations of 11.5% and 7.4%, respectively. There were two optimum treatment combinations: 11.5% maltodextrin at 80^{0}C and 7.4% glucose syrup at 60 ^{0}C.

Keywords: Papaya powder; Rehydration; Spray drying; Carrier agent; Drying temperature

Introduction

Papaya (*Carica papaya* L.) is an inexpensive fruit that belongs to the Caricaceae family and is available all year round, regardless of the season or weather. It is native to Mexico and northern South America but has become naturalized in many parts of the world, including tropical and subtropical regions (Sharma *et al*., 2020). Due to the obvious plant's economic and nutritional potential, it has become a popular fruit and vegetable. *C. papaya* is an excellent source of vitamins A and C (Atintunde *et al*., 2022). Thiamine, riboflavin, calcium, iron, potassium, magnesium, and sodium are all included in small quantities (Admad *et al*., 2013). Antioxidant content of papaya fruits is considerably high according the study conducted by Maisarah *et al*., (2013).

The recent studies revealed that papaya post-production losses are between 25%-40%, particularly in developing countries (Gunathilake *et al*., 2018). Mechanical damages during harvesting, transportation, and post-harvest diseases cause significant loss of papaya fruits. Several diseases, primarily of fungal origin, affect papaya fruits as they ripen, and a considerable percentage of fruits are lost after harvest. According to the Samithri *et al*. (2020), Phomopsis rot (*Phomopsis caricae-papayae*), stem-end rot (*Lasiodiplodia theobromae*) and Anthracnose (*Colletotrichum gloeosporioides*) are the most common postharvest diseases of papaya in Sri Lanka, causing up to 45% postharvest losses (Sarananda *et al*., 2004).

However, there is a growing interest in reducing these losses by processing fruits into value added products. Fruit juice powders provide several advantages and economic potentials over liquid alternatives; such as lower weight or volume, reduced packaging, better handling, transportation, and a

considerably longer shelf life (Gomes et al., 2018). Dehydration is an effective way for fruit preservation since that decreases the water activity of fruits, which suppresses microbial development and enzymatic activity. According to of the literature revealed literature foam-mat drying, freeze drying, hot air oven and solar drying were used to produce papaya powder but limited studies in papaya powder production by spray drying (Chang *et al*., 2020).

Spray drying is one of the most used methods for producing heat-sensitive fruits and vegetables. The physicochemical characteristics of the powders are mostly determined by the operational conditions, carrier agent usage, and respective concentration. Lower amount of viscosity and homogenous consistency of feeding reduces clogging during drying and enhances spray drying effectiveness (Bazariya and Kumar, 2018; Gomes *et al*., 2018). A carrier agent is a substance that acts as a physical barrier and wall to protect functional ingredients from unfavorable environmental conditions. These materials improve the stability of active substances while maintaining product quality. An ideal carrier agent must have the following properties: adequate rheological properties (low viscosity) at high concentrations and simplicity of handling while processing, chemical affinity to disperse or emulsify the bioactive substance, as well as the ability to stabilize the emulsion produced, unreactive with the bioactive material during the drying process or storage conditions, ability to capture and retain the substance to be contained inside its structure; and capacity to shield the active substance from environmental conditions to the greatest extent possible (temperature, amount of air and humidity) (Wisniewski, 2015). Conventional spray drying technologies mainly influence end product characteristics of the dried fruit powders; including moisture content, solubility, vitamins, pH, yield and also product quality (Stavra *et al*., 2022).

The purpose of this study was to assess the feasibility of using spray drying technology to produce reconstitutable fruit juice powder. The current study aims to find the best carrier agent and better processing temperature with minimal impact to the available nutrient components in papaya spray drying process. The research findings will be beneficial to the commercialization of the papaya spray drying technology in Sri Lanka.

Materials and methods

Methodology was consisted of three steps. Such as sample preparation, homogenization and spray drying.

Sample preparation

The fruits were purchased from outlet of the A-farm Gannoruwa. Fruits were cleaned before being scraped with a spoon and weighed by balance. Pulp was extracted and blended with water, strained through stainless steel strainer to remove seeds and fibrous matter. Maltodextrin and Glucose syrup used as desirable carrier agent which assistance to transfer, drying and reconstitution of product. The flesh sample was then treated with a carrier agent (Maltodextrin or Glucose syrup). Following that, a carrier agent (Maltodextrin or Glucose syrup) was weighted and applied to the flesh sample.

Homogenization

A homogenizer was used to homogenize the prepared mixer. A homogenizer can be used for big quantities, while a high-speed blender is preferred for small quantities.

Spray drying

Prior initiating the operation, the spray dryer was switched on, and then allowed to attain an internal temperature to reach 100 °C. After reaching the desired temperature, the atomizer was turned on, and then sample was fed into the spray drier via atomizer's feed inlet. When the process was completed, the atomizer and other switches were turned off. End product was obtained from the bottom as well as the cyclone collector and then it was packed air tightly.

Product development trials

Two set of trials were conducted to determine suitable temperature and ratios for spray drier. In first set of trials, spray drying of papaya was conducted at three different temperatures as 60 °C, 80 °C and 100 °C. Same as previous other set of trails conducted with Glucose syrup in different ratios. The dry matter of the fruit pulp determines the percentage of carrier agent applied. The pulp-to-

maltodextrin and pulp-to-glucose syrup ratios were given in Table 1. The ideal ratio of fruit pulp to maltodextrin and pulp Glucose syrup was chosen for product analysis and future investigations based on solubility, color, and reconstitutability (Lacerda *et al*., 2016).

Table 1: Pulp-to-maltodextrin and pulp-to-glucose syrup ratios

Sample No	Pulp to Maltodextrin (g)	Pulp to Glucose syrup (g)
1	100:8	100:8
2	100:13	100:13
3	100:18	100:18

Physio-chemical properties of papaya powder

Solubility

Solubility was determined according to the method used by Cano-Chauca *et al*. (2005). A dried, 50 mL centrifuged tube was filled with 2 g of papaya powder and 10 mL of water, mixed thoroughly to make a homogenous, lump-free paste and volume it up to 25 mL. The mixture was vigorously shaken for 3 minutes. The tube was centrifuged for 15 minutes at 4000± 100 rpm, and after that solution was put into a petri dish and was oven dried. Then weight differences of dish were used to measure solubility (%) by mass.

Dissolved solids

The 5 mL of solution was weighed with its contents and then placed in a boiling water bath for 15 minutes and then oven dried at 103 °C for 3 hours (Jazaeri, 2009).

Determination of residues

The residual solution was decanted off as thoroughly as possible without disturbing the sediment at the bottom of the tube. Contents of the tube were dried using boiling water bath and then 103 °C oven for 3 hours. The weight was taken in hourly intervals up to constant weight obtained (Yang *et al*., 2019).

Rehydration time

Powdered product 1 g was added into 50 mL of distilled water at room temperature (28°C). The mixture was agitated on a magnetic stirrer at 550 rpm until the powder disappears. The time taken for the powder to be completely disappearing was recorded.

Vitamin C (Ascorbic Acid)

The ascorbic acid content was measured using 2, 6-dichlorophenolindophenol as explained the AOAC (1995) and the content was calculated using the following equation (1).

$$Ascorbid\ acid\ (mg/100g) = \frac{Titre \times Dye\ factor \times Volume\ made\ up \times 100}{Aliquot\ of\ extract\ taken \times \text{Weight of sample}} \quad \text{Equation 1}$$

Titratable acidity

Titratable acidity was calculated by titrating a known amount of powder (3 g) against a standard solution of 0.1 N sodium hydroxide, with phenolphthalein indicator. The findings were represented as a percentage of citric acid (Ranganna, 1986) and calculated using equation 2.

$$\%\ Titratable\ acidity = \frac{Titre \times \text{Normality of alkali} \times \text{Equivalent weight of acid} \times 100}{Volume\ of\ aliquot\ taken \times \text{Weight of sample taken} \times 100} \quad \text{Equation 2}$$

Determination of moisture content

The moisture content was calculated using the AOAC method (1995). A 2 g sample was weighed and added into pre-weighed and pre-dried moisture cans and dried at 103 ^{0}C for 6 hours. Then the dry samples were placed in a desiccator and cooled to room temperature. Then weighed, and the moisture content in percent was calculated using equation 3.

$$Per\ cent\ moisture = \frac{Loss\ in\ weight \times 100}{Weight\ of\ sample} \quad \text{Equation 3}$$

pH

A 2 g of measured sample placed in a beaker and it was dissolved in 25 mL of distilled water. Then the reading was obtained using pH meter (model RM-131 Hand Held).

Sensory evaluation

Sensory evaluation was conducted to determine the best processing temperature and carrier agent concentration for maltodextrin and glucose syrup, based on chemical and physical property studies. Sensory analysis was conducted using a 30 untrained panel in order to assess whether there are significant differences among samples.

Statistical analysis

The experiment data were analyzed using one-way ANNOVA, and mean comparisons were done using LSD. The SAS version 9.1.3 (2007) computer software was used for analysis of data with a significance level of $p \leq 0.05$.

Results and discussion

Physicochemical properties

Solubility

Moisture content has the greatest influence on solubility as moisture content increases, solubility decreases with the moisture content (Grabowski *et al*. 2008). This could be due to a hard surface layer formed over the powder particle, preventing water molecules from diffusing through the particle as a result of very high inlet temperature (Fazaeli *et al*., 2012). According to the Cano- chauca *et al*. (2005) when the temperature reaches 100 °C, the caramelization of sugar in papaya fruit is affected. The solubility of spray dried papaya powder is reduced as a result of caramelization. Figure 1 depicts the physical property analyses of spray-dried papaya powder at different temperatures and carrier agent concentrations. According to the results shown in Figure 1a and 1b solubility of spray dried papaya powder was ranged from 18-31% for maltodextrin and 17-31% for glucose syrup carriers. Although,

considering 60 ^{0}C solubility increased with increasing temperature in both glucose syrup and maltodextrin. But with 800 ^{0}C and 1000 ^{0}C temperatures it shows decreasing trend. Also, concentration of both maltodextrin and glucose syrup has no significance difference ($p \leq 0.050$).

Rehydration time

The rehydration time indicates the speed with which sprays dried powder reconstitute (Sathyashree *et al.*, 2018). The speed of reconstitution is proportional to the moisture content of the processed powder. High moisture content has a high tendency for agglomeration, which helps to boost the powder's reconstitution speed and shorten rehydration time (Jinapong *et al.*, 2008). According to the results shown below Figure 1c and 1d the range of results for maltodextrin and glucose syrup were 2.75 - 5 min and 3.23 - 5.09 min respectively. However, maltodextrin concentration is inversely proportional to rehydration time whereas the concentration of glucose syrup increases rehydration time. According to the results of statistical study, there is a significant difference in rehydration time between maltodextrin and glucose syrup as a carrier agent ($p \leq 0.05$).

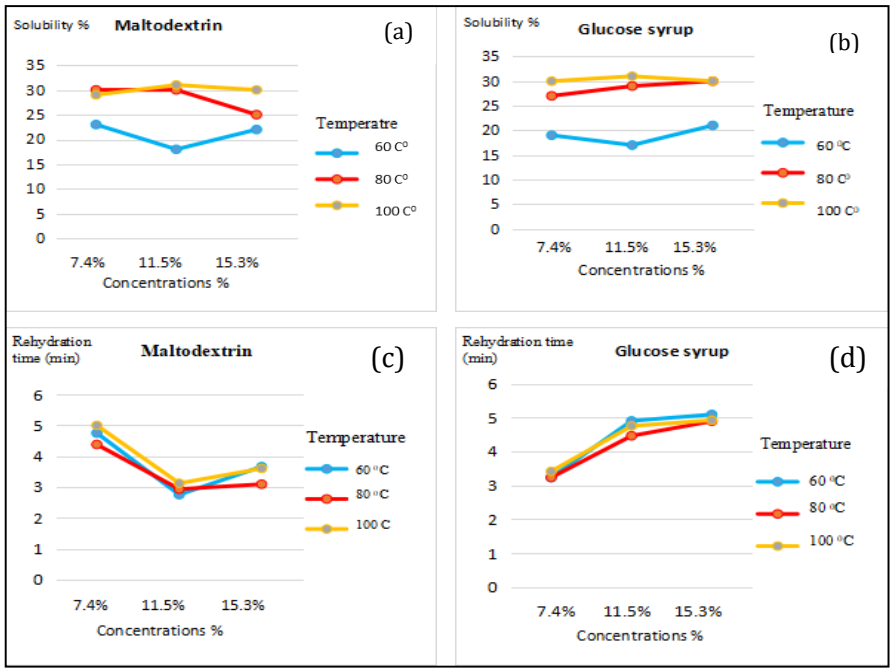

Figure 1: Physical properties of spray-dried papaya powder at different temperatures and carrier agent concentrations; (a) solubility with maltodextrin; (b) solubility with glucose syrup; (c) rehydration time with maltodextrin; (d) rehydration time with glucose syrup

Effect of moisture content with carrier agent concentration and temperature

The moisture content (MC) of papaya powder decreased as the maltodextrine concentration increased. The highest and lowest MC was found in papaya powder samples with maltodextrine concentrations of 7.4% and 15.3%, respectively. A similar finding is reported by Mishra *et al*. (2014). According to the Suhag *et al*. (2016), at higher temperature, the moisture content of the powder particles decreased. This might be attributed due to the faster rate of heat transfer into the particles at high temperatures, resulting moisture removal efficiency, in spray drying. It is also reported that as the drying temperature increases, the moisture content of apple juice powder, pomegranate juice, and lemon juice, decreased (Minim *et al*., 2009; Michalska, 2018; Jafari *et al*., 2017). The moisture content did not significantly change with temperature (60 °C, 80 °C, or 100 °C) at given concentration of maltodesxtrin. According to the Figure 2, the MC increases with increasing glucose syrup concentration up to 11.5% at 60 °C and 100 °C, then decreases with increasing glucose syrup concentration. There is a significant difference in moisture content between maltodextrin and glucose syrup as carrier agents.

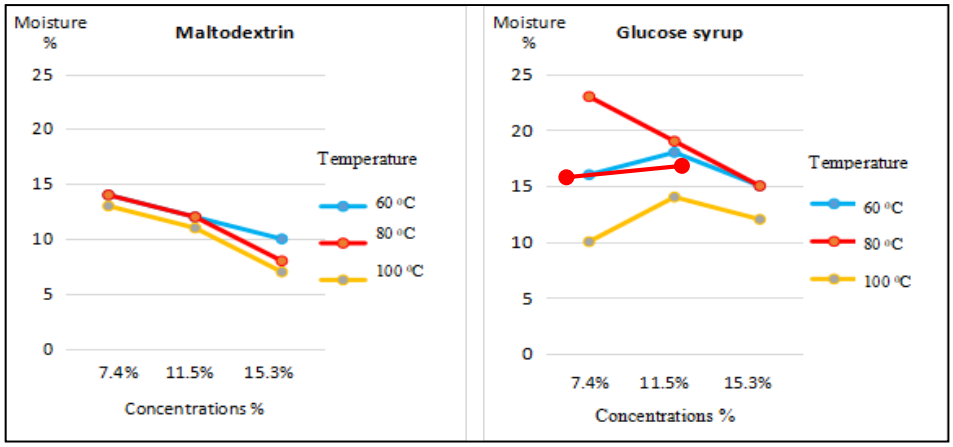

Figure 2: Effect of temperature and concentration of maltodextrin and glucose syrup on moisture content of spray dried papaya powder

Ascorbic acid

Spray dried papaya powder contains a small amount (0.55 mg/g) ascorbic acid (Surekha *et al*., 2018). Because of the heat sensitive features of ascorbic acid, the amount of ascorbic acid decreased as the temperature increased (Islam *et al*., 2016). Thankitsunthorn *et al*. (2019) reported that, at 140 °C, typical spray drying of gooseberries juice resulted in a 62.1% loss of vitamin C and around 28-51% of vitamin C losses at temperature between 220 °C to 250 °C from spray drying of cactus pear powder ((Rodríguez-Hernandez *et al*., 2005). Angel *et al*. (2009) shown that 27.47% of vitamin C was maintained throughout the drying process of kiwifruits without a carrier agent at 60 °C. The proportion of vitamin C maintained during spray drying of passion fruit with matodextrin at 180 °C and 190 °C ranged between 39.73% and 56.89%. This indicates that vitamin C is extremely heat and oxidation sensitive.

In this study the lowest ascorbic acid (0.09 mg/g) was observed in 11.5% of maltodextrin and glucose syrup concentrations at 100 °C (Figure 3). Highest ascorbic acid content (0.17 mg/g) was observed in glucose syrup carrier agent at 60 °C. According to the results, there is no statistically significant difference (P>0.05) between maltodextrin and glucose syrup as a carrier agent for content of ascorbic acid for three different temperatures.

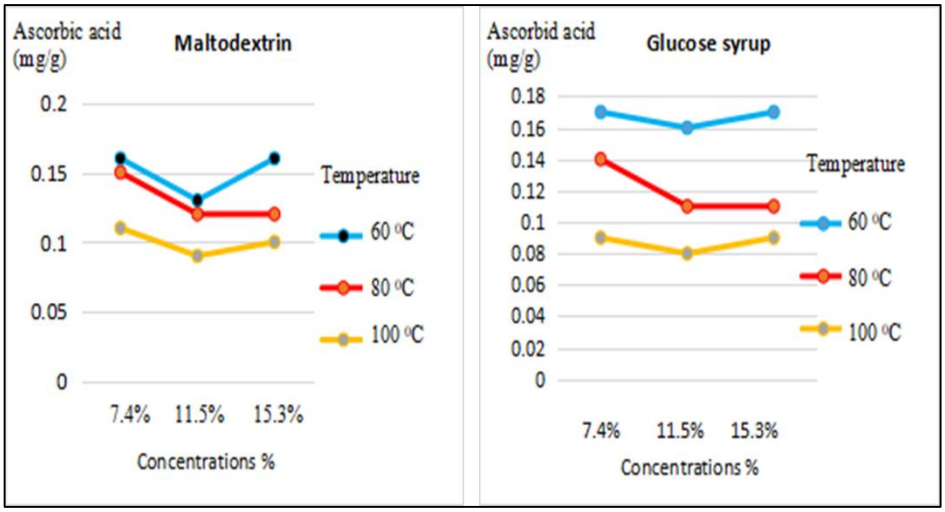

Figure 3: Effect of temperature and concentration on ascorbic acid of spray dried papaya powder with maltodextrin and glucose syrup carrier agents.

pH

The acidity level of the spray dried papaya powder has the greatest influence on pH. Spray dried powder has a better pH level of around 5 (Chang *et al*., 2020). According to the results which are depicted in Figure 4 maltodextrin with a concentration of 15.3% had the highest pH value at 100 °C. The highest pH was observed in 15.3% of glucose syrup as a carrier agent at 80 °C. The pH range of maltodextrin and glucose syrup was 4.9-5.3 and 4.9-5.4 respectively within all temperature levels. And there is no significance difference between pH for both maltodextrin and glucose syrup carrier agents (P>0.05).

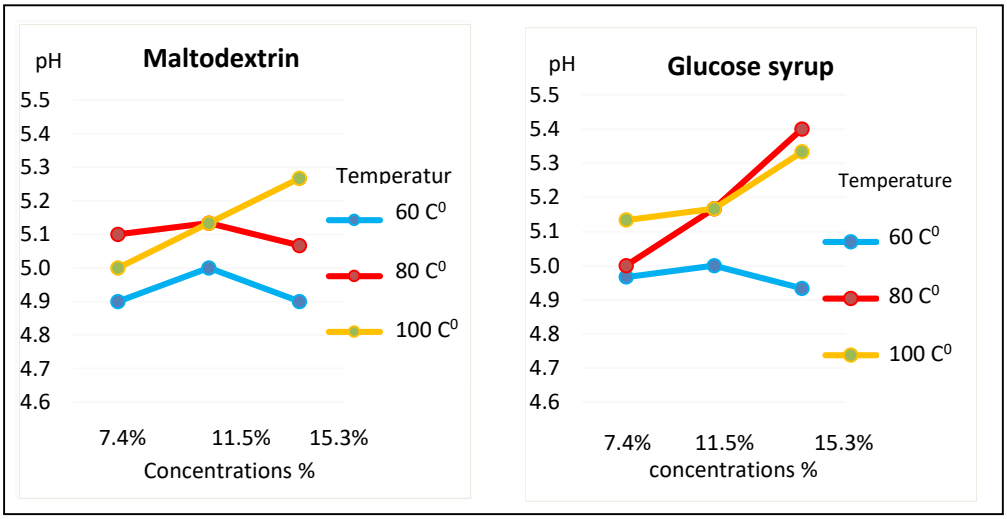

Figure 4: Effect of temperature and concentration of maltodextrin and glucose syrup on pH of spray dried papaya powder

Titratable acidity is an approximation of the solution's total acidity and is critical in the food processing sector. Further it has an impact on the shelf life period of the processed food. Commercially, a titratable acidity range of 0.5 to 0.8 is considered desirable (Karel & Lund, 2003). According to results shown in Figure 5, pH ranged from 0.53-0.89 for maltodextrine and 0.35-0.78 ± 0.12 for glucose syrup. When the concentration of maltodextrin was increased, the titratable acidity reduced. Consider glucose syrup as a carrier agent; it has the maximum titratable acidity at 11.5% concentration at all three temperatures. According to the results of statistical investigation, there is a significance

difference in titratable acidity between maltodextrin and glucose syrup as a carrier agent (P>0.05).

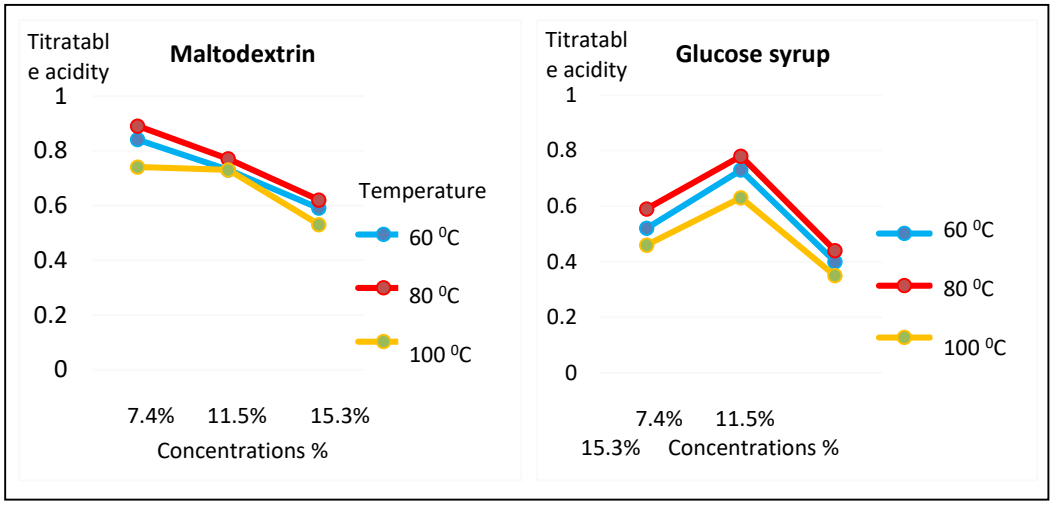

Figure 5: Effect of temperature and concentration on titratable acidity

Mean separation

According to the results shown in Table 2, there is a significant difference between three concentration levels of maltodextrin and different temperature levels. Maltodextrin concentrations of 11.5% and 15.3% received the greatest ranking value of 0.26 and the lowest ranking value of 0.17, respectively. Temperature levels of 80 °C and 100 °C had the highest ranking value of 0.17 and the lowest ranking value of 0.11. According to the results, a maltodextrin concentration of 11.5% at 80 °C shows a superior condition for the papaya spray drying process.

Table 2: Mean separation of maltodextrin

Concentration	Mean Rank	Grouping	Temperature	Mean Rank	Grouping
7.40%	0.21	B	60°C	0.14	B
11.50%	0.26	A	80°C	0.17	A
15.30%	0.17	C	100°C	0.11	C

According to the results in Table 3, there is a significance difference among three concentration levels of glucose syrup and different temperature levels. According to the results, glucose syrup concentrations of 7.40% and 15.3% had the greatest ranking value of 0.25 and the lowest ranking value of 0.13, respectively. Temperature levels of 60 °C and 100 °C had the highest ranking value of 0.17 and the lowest ranking value of 0.09. According to the results, a glucose syrup concentration of 7.40% at 60 °C is a superior condition for the papaya spray drying process.

Table 3: Mean separation of Glucose syrup

Concentration	Mean Rank	Grouping	Temperature	Mean Rank	Grouping
7.40%	0.25	A	60°C	0.17	A
11.50%	0.14	B	80°C	0.1	B
15.30%	0.13	C	100°C	0.09	C

Sensory evaluation

The best processing temperature and carrier agent concentration for maltodextrin and glucose syrup were determined based on chemical and physical properties. The sensory assessment result for the selected carrier

agent concentration discovered by chemical and physical study for different temperatures is shown in Figure 6. Selected concentrations were 11.5% and 7.4% respectively maltodextrin and glucose syrup as suitable for spray drying.

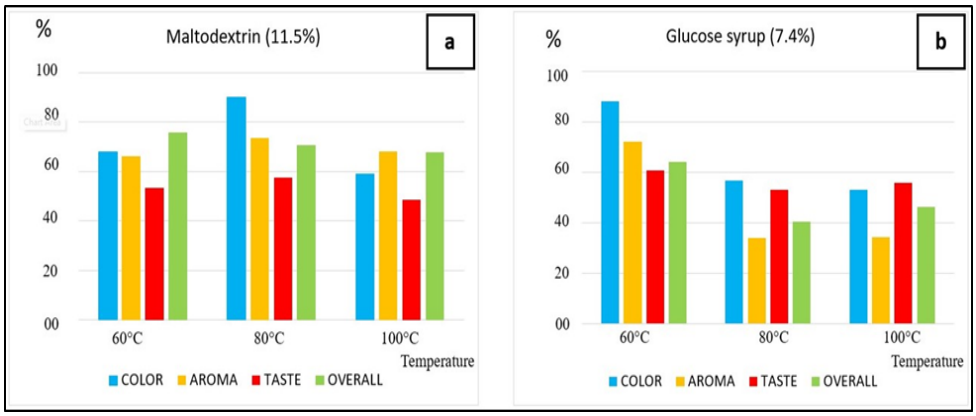

Figure 6: Sensory evaluation results; (a) maltodextrin concentration of 11.5%; (b) glucose syrup concentration of 7.4%

The sample processed with maltodextrin at 80 °C had higher sensory test scores than the other two temperatures. Color, aroma, and taste were scored at 80 °C with 90%, 74%, and 58%, respectively (Figure 6a). The sample processed with glucose syrup at 60 °C had higher sensory test scores than the other two temperatures. Color, aroma, and taste were scored at 60 °C with 88%, 72%, and 60%, respectively (Figure 6b).

Conclusion

According to findings of the study, Maltodextrin is the best carrier agent for the papaya spray drying method. The best processing temperature and the best Maltodextrin concentrations are 80 °C and 11.5% respectively. A comparable outcome may be accomplished with Glucose syrup at 60 °C and a concentration of 7.4%. When the processing temperature is raised, most of the nutritious components and physical properties of the food are deteriorated. Because keeping the temperature between 60 °C and 80 °C is best for preventing damage. If the temperature rises beyond 80 °C, the sugar in the fruit juice caramelizes, lowering the quality of the papaya powder. The sensory test results

show that papaya reconstitutable powder may give acceptable sensory characteristics at 80 °C with Maltodextrin and 60 °C with Glucose syrup.

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Enhancement of lipid yield in *Chlorella* sp. under different stress conditions for the production of biodiesel

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Abstract

Microalgae derived bio-lipids can be potentially converted to biofuel using appropriate technologies. However, optimum conditions for enhancing the growth of microalgae often lead to relatively low accumulation of bio-lipids. Alternatively, stress conditions favor the storage of lipids despite the low biomass yield. For commercial production of microalgae derived biofuel, the unit cost of production of biodiesel should be lower than that of petro diesel. The aim of the present study was to enhance the lipid yield of *Chlorella* sp. by introducing various stress conditions such as nitrogen deficiency, zinc deficiency and excessive salinity in the growth media. Conventional Bold's Basal Media (BBM) was used as the culture media in all the experimental conditions. Five treatments were used namely; normal Bold's basal media, three times concentration enhanced Bold's basal media, Nitrogen deficient media, Zinc deficient media and Salinity stress media. Light intensity was kept constant in all the experimental conditions.

Citation:
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Air mixing was provided using spargers in all test conditions to keep the microalgae cultures in suspension. All the reactors were maintained in similar growth conditions in the first phase and the stress conditions were introduced in the second phase after 7 days. The microalgae biomass was harvested on 4th, 8th, 11th, 14th and 17th day of cultivation for the determination of lipids. A Chloroform: Methanol mixture with 1:1 ratio was used to extract lipids from dried biomass using Soxhlet extractor. A considerable increase in the lipid content was observed in all the test conditions compared to control samples (highest was 16.9%). Highest lipid content of 23.3% was observed in Zn stressed conditions. However, in terms of lipid productivity, salinity stress treatment produced the highest average lipid productivity of 0.008±0.005 $mgL^{-1}d^{-1}$. After certain period of time (14th day), lipid content of stressed samples decreased. Oleic acid and linoleic acid were found to be abundant in all of the treatments. The study suggests that imposing stressful conditions can result in a higher amount of lipid yield from the algae biomass with a higher rate of productivity that contains important fatty acids which can be a feasible opening for biofuel production.

Keywords: *Chlorella* Sp.; Stress Conditions; Lipid Production; Lipid productivity; Biodiesel

Introduction

Microalgae can produce their own food by utilizing inorganic raw materials from the surrounding environment. Years of research have resulted in a significant amount of interest worldwide due to their potential application in renewable and sustainable sources of biofuels, bioactive medicinal products, and food sources. Several microalgae species have been studied for their potential as value-added products with exceptional pharmacological and biological properties (Khan *et al*., 2018). With an ever-increasing global population, the demand for food and energy sources is increasing proportionally, but resources, particularly energy resources, are degrading inversely proportionally to the global demand. As a result, long-term solutions are required for these problems. On the other hand, the use of fossil fuels and improper disposal of end products have gradually annihilated the environment to the point where it is becoming increasingly toxic for all living beings to consume.

As a result, there is a growing demand for environmentally friendly energy and food resources. Phototrophic microalgae require light energy, carbon source and other inorganic compounds including nitrogen (N), phosphorus (P), iron (Fe) for their growth and maintenance. Restriction of nutrients can have an impact on the lipid composition of microalgae cells (Tsai *et al*., 2016). The accumulation of fatty acids and lipids in the microalgae body typically occurs during times of environmental stress (Sajjadi *et al*., 2018; Renuka *et al*., 2018). Green algae cells typically undergo metabolic acclimatization in response to nutrient stress, which always results in macromolecule cellular composition fluctuations. Nitrogen deficiency frequently leads to decreased protein content and increased carbohydrate or lipid storage (Lin , 2011). Phosphate restriction also has a significant impact on protein, lipid, and carbohydrate content. As a result, green alga's biochemical configuration is linked to its growth rate which reflects the physiological potential of primary productivity. Microalgae cells that are deprived of nutrients can change chemically. It can have an impact on the growth rate and photosynthetic efficiency. It has been demonstrated that changes in cellular composition improve their ability to cope with physiological parameters. Protein synthesis in chlorophytes is limited by nitrogen limitation because carbon is inserted into the formation of storage products such as carbohydrate and lipids (Minhas *et al*., 2016).

The response of microalgae in terms of particulate organic carbon, chlorophyll, and cell concentrations demonstrated that nitrogen is the better principal limiting nutrient than silicon (Delgadillo-Mirquez *et al*., 2016). In this study, nitrogen limitation retarded the growth of microalgae and led to accumulation of lipids due to algae's biosynthetic response to environmental changes. Li *et al.* (2008) reported that different microalgae species stimulate different levels of neutral lipid accumulation. Although microalgae can produce lipids and other nutrients that can be used to produce energy in the form of food or fuel, the amount they produce has not been found to be sufficient to meet global energy demands. As a result, various techniques and approaches to increasing the rate of production are being investigated.

Chlorella Sp. is one of the microalgae species that has been studied for its ability to accumulate a high amount of lipids under various stress conditions such as: light intensity; temperature; carbon dioxide; nutrient deficiency; salinity stress

and metal stress during cultivation (Zhu *et al*., 2015). The potential to produce biolipids through *Chlorella* Sp*.* has been found to be important due to the ability of *Chlorella* Sp to to grow under different conditions. In light of this, the current study aimed to enhance the lipid content of *Chlorella* Sp*.* by applying different conditions such as: nitrogen; Zn and salinity stress under laboratory conditions.

Materials and methods

Selection and culturing of microalgae strain

The model microalgae for the series of experiments were a culture of *Chlorella* Sp. (Obtained from the Environmental Microbiology Laboratory, Department of Civil Engineering, University of Jaffna). The chosen *Chlorella* Sp. mix culture was grown in clear 20 L plastic bottles and served as the base culture for the following experiments. The medium for algal growth was laboratory prepared Bold's Basal Medium (BBM) (Aragaw & Asmare, 2017). To prepare BBM, a standard procedure was followed (Aragaw & Asmare, 2017), and all of the stock solutions and trace elements used to prepare Bold's Basal Medium (BBM) were obtained from Sigma Aldrich, USA, Sri Lanka. All of the chemicals used were of the highest analytical grade. The prepared stock solutions and chemicals were kept in a refrigerator set to 4 ˚C (Anon *et al*., 1992). The pH of the finished BBM was adjusted to 6.6. All glassware was autoclaved at 121 ˚C for 30 minutes before use in all procedures.

Preparation of polybag Photo Bioreactor (PBR)

The photo bioreactors were built with thick, transparent polythene. The 150 mm wide, 500 mm long polythene was sealed at both the top and bottom ends to create a 400 mm x 150 mm working area polythene bag photo bioreactor. An opening in the bag's top was made to accommodate the air sparger, testing culture, and other treatments. It was hung in the metal setup, a lighting system that provides continuous 2000 lux white fluorescent light. The aquarium air pump was used to aerate the photo bioreactor, and porous air stones were used for mixing. The photo bioreactor had a total volume of 2.5 L.

Applying stress conditions on microalgae culture

To provide stress conditions, a mixed culture of microalgae (*Chlorella* Sp.) was grown as batch cultures using five (05) different treatment methods with

different amounts of growth medium and varying certain nutrients according to the labels. As tabulated in Table 1, the treatments were labeled as BB, BBx3, N, Z, and S.

Table 2: Treatments that have been used and their symbolic labelling

Treatment	Label
01. Normal BBM	BBM
02. 03 times strength increased BBM	BBMx3
03. Nitrogen stress	N
04. Zinc stress	Z
05. Salinity stress	S

As illustrated in Figure 1, each treatment had 05 replicates that were harvested at various points throughout the study. The algal biomass was extracted from the base culture using a refrigerated centrifuge (10000 rpm for 2 minutes at 4 ˚C). The harvested biomass was re-suspended in photo bioreactors that had been previously prepared (25 polythene pouches). The initial optical density was kept constant at 0.200 at 680 nm (OD_{680}).

Treatment 01 was designated as BB, and it included 05 replicates (BB-1, BB-2, BB-3, BB-4, and BB-5) containing microalgae biomass (*Chlorella* Sp. mix culture) and an appropriate amount of BBM. To prevent nutrient deficiency, the BBM was reinstalled in treatment 01 after 07 days from culture start (on the 8th day). This treatment served as the initial control set. The second treatment (Treatment 02) was called BBx3, and it contained three times the recommended amount of BBM. The same reinstalling procedure and replicates (BBx3-1, BBx3-2, BBx3-3, BBx3-4, and BBx3-5) were used as in the first treatment. The second control set was Treatment 02.

Treatment 03 was used to test microalgae growth and lipid production in N deficient medium. The treatment was labeled N, and there were 05 replicates (N-1, N-2, N-3, N-4 and N-5). In the absence of sodium nitrate, an appropriate amount of BBM was added to each PBR to simulate nitrogen stress conditions. To avoid any nutrient deficiency, sodium nitrate less BBM was added again on the eighth day of the culture in the same manner as before. Treatment 04 was

designed to put the Zinc (Zn) stress to the test. As a result, it was labeled Z, and 05 replicates were included (Z-1, Z-2, Z-3, Z-4 and Z-5). Except for the Zn present in BBM, all of the Zn bioreactors received an adequate amount of BBM. After 7 days, the same Zn-less BBM was reinstalled in the culture (on 8th day).

Salinity stress has been shown in previous studies to improve total lipid content and saturated portions of fatty acids (Church *et al*., 2017), therefore the 5th and final treatment was added an extra amount of NaCl (2M) to a PBR containing an appropriate amount of BBM. S was the name given to treatment 05, which had 05 replicates (S-1, S-2, S-3, S-4 and S-5). On the eighth day, each salinity stress replicate received the same extra amount of NaCl (2M) and the appropriate amount of BBM. The 25 PBR were arranged in a random manner in the pre-made metal setup as mentioned in Figure 1. Random sampling was used to determine microalgae growth in the treatments using OD680 and a UV visible spectrometer (Lovibond XD, 7500). Daily samples were collected for analysis.

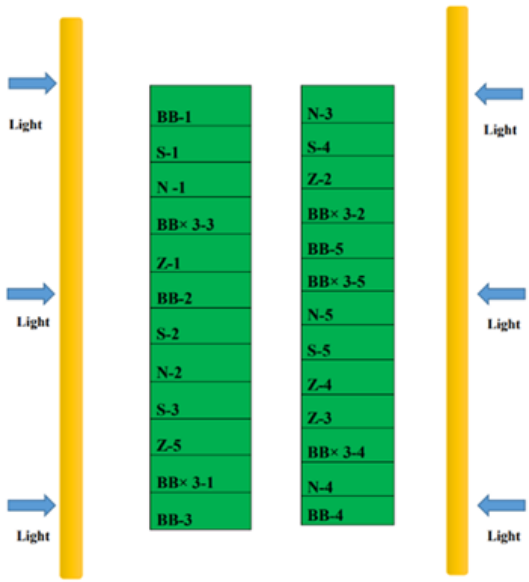

Figure 1: Placement of each photo bioreactor in experimental setup

Microalgae biomass harvesting and preparation

To obtain algal precipitation, Chlorella Sp. culture samples were centrifuged (under 10000 rpm at 4 ^{o}C for 2 minutes) (pellet). Wet algal biomass was obtained through centrifugation. Wet algal biomass was collected in petri dish after supernatant was removed from centrifuge tubes and dried in an oven at 60^{o}C until dry biomass was obtained. Lipid was extracted from dry biomass. A random sample from each treatment was taken. One sample pouch was randomly selected from each treatment on each day of harvesting (As there were 5 treatments, 5 samples were harvested). Harvesting was done on the 4th, 8th, 11th, 14th and 17th day since the start of the experiment.

Determination of biomass productivity

Samples of 50 mL from each treatment were collected and filtered through pre-weighed 45 m Whatman glass fiber filter papers. It was then rinsed three times with 20 mL deionized water. The sample was then dried at 105℃ until it reached a constant weight. Using dry weight measurements, the biomass productivity was calculated using the following equation. (Ambat *et al*., 2019)

$$Biomass\ productivity\ (gL^{-1}d^{-1}) = {Biomass\ concentration\ (gL^{-1})}\Big/{No.of\ days}$$

Equation 2

Lipid extraction from dry microalgae biomass

For lipid extraction, a Büchi B 811 Soxhlet (BÜCHI Universal Extraction System, Switzerland) apparatus was used. The continuous flow method was used (Ramluckan *et al*., 2014), and the extraction took 3 hours. According to the preliminary test results, the solvent system was chloroform: methanol (1:1, v/v). Following extraction, the lipid percentage was calculated using the equation;

$$Lipid\ percentage = \frac{M_L}{M_B} X\ 100$$ Equation 2

Where M_L = Mass of lipid extracted (g), M_B = Mass of Biomass weighted (Dry) (g).

The lipid productivity was calculated using following equation from (Ambat *et al*., 2019)

$$Lipid\ productivity\ (mgL^{-1}d^{-1}) = biomass\ productivity\ (mgL^{-1}d^{-1}) \times Lipid\ content\ (\%)$$

Equation 3

Trans-esterification of extracted lipids

Toluene (2 mL) was used to dissolve the extracted lipids. The contents were then transferred to a screw-capped glass test tube lined with Teflon (20 mL capacity). A 7% BF_3-methanol reagent of 2 mL was added. BF_3 is used as an acid catalyst (Ichihara and Fukubayashi, 2010). The Teflon-lined screw-cap was used to seal the glass. The tube was heated in a heating block, oven, or hot water bath for 45 minutes at 100 °C. Every 10 minutes, the tube was gently shaken (Note: evaporation of solvent from tubes indicates inadequate seals. If this happens, discard the solution and repeat the methylation procedure). After removing the tube from the heating block (oven or hot water bath), it was allowed to cool to room temperature. There was 5 mL distilled water, 2 mL hexane, and 1 g sodium sulphate added. The tube was sealed and shaken. Fatty acid methyl esters (FAMEs) were collected in hexane solution to a small seal glass vial after 10 minutes. Filled with nitrogen and secured the cap. The sample was immediately analyzed on GC (Lohman *et al*., 2013 and Griffiths *et al*., 2010).

Gas chromatography analysis

FAMEs were determined by injecting 1 µL into a GC (Agilent technologies) system outfitted with a Flame Ionization Detector and a fused silica capillary column (100 m length, 0.25mm diameter, and 0.20 m film). At a flow rate of 20 mL/min, nitrogen was used as the carrier gas. The split was 30:1. Operating parameters for the GC: The initial column oven temperature was maintained at 140 ^{o}C for 5 minutes before increasing to 240^{o}C for 10 minutes at a rate of 4 ^{o}C/min and remaining at that temperature for 10 minutes. The injection port temperature was kept at 260 ^{o}C, and the detector temperature was kept at 260 ^{o}C (Ambat *et al*., 2019).

Results and discussion

Microalgae growth behavior

The growth of *Chlorella* Sp. under five different treatments is shown in Figure 2. The *Chlorella* Sp. mix culture exhibited substantial growth in all treatments based on the variation of optical density over 17 days. The algal strains exhibited a two-day lag phase in each of the treatments (Ambat *et al*., 2019). However, among other treatments, the Zinc stress media (Treatment 04) exhibits a distinct increase in growth. This finding contradicts with a previous study (Hamed *et al*., 2017), in which the author reported that zinc stress inhibits growth and induces oxidative stress.

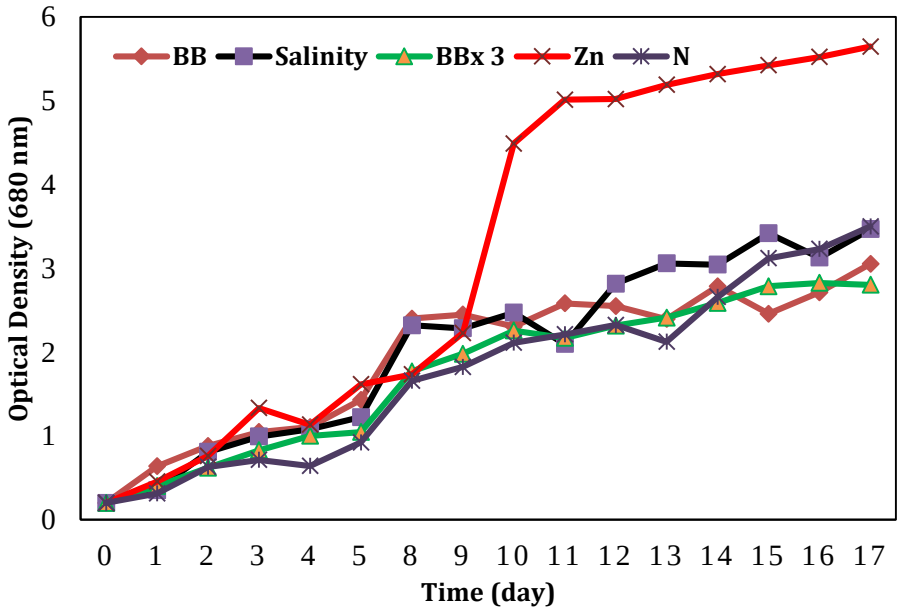

Figure 2: Growth of Chlorella Sp. microalgae in all treatment conditions

For the treatment with the appropriate amount of BBM, the optical density increased rapidly on the first day and gradually increased from the second to the fifth day. From the fifth to the eighth day, the optical density increased rapidly again, and from the eighth day onwards, the parameter had a pattern of rapidly increasing and decreasing.

For the treatment of salinity, the behavior is nearly identical to that of BB treatment. However, it increased slightly on the first day, and from the ninth to the sixteenth day, the salinity variation is opposite to the BB treatment. When considering the treatment of three times the concentration of BBM, there is a slight increase from the first day to the fifth day, and a rapid increase between the fifth and eighth days. Then it gradually increases and decreases until it reaches a constant value. The variation in the treatment with nitrogen stress is almost identical to the variation in salinity, with a small deviation up to the twelfth day, when it rapidly increased. Previous research confirmed that nitrogen deficiency can reduce biomass growth rate, but nitrogen addition at specific time intervals can keep biomass growth at an optimal level (Zhu *et al*., 2015). The ability of *Chlorella* Sp. to thrive in various conditions throughout these experiments in various treatments suggests that *Chlorella* Sp. has the potential to be used as a better option for nutrient removal in a variety of wastewaters.

Biomass production

The observed weight of biomass under all the treatments is shown in Figure 3. Almost in all the treatments, 3rd day of harvesting resulted in twice the weight of biomass which were harvested in the 2nd harvesting day possible due to the addition of nutrients again in the 8th day of the experiment. An exponential biomass growth was observed in the BBx3 treatments because of the presence of nutrients 3 times more than the standard Bold's basal medium (BBM). The recorded highest biomass yield from Zinc stress treatment and Nitrogen stress treatment came on 3rd and 4th harvesting days respectively (11th and 14th days of the experiment). All other treatments recorded their highest biomass yield on the final day of the harvesting which indicates that deficiency of Zinc and Nitrogen have an effect on the production biomass in *Chlorella* Sp. microalgae. A study by Chandra *et al*. (2019) states that Zinc is an essential micronutrient for microalgae growth, therefore, the lack of Zinc results in a weaker growth in microalgae. A recent study suggests that the deficiency of Nitrogen suppresses the growth of *Chlorella* Sp. microalgae (Ratomski *et al*., 2021). All these observations are in line with the findings of the present study. The prediction of cell density by $OD_{680}$was not always in good agreement with the actual cell

density measurements. This is presumable due to the formation of microalgae flock in the bioreactor despite the gas mixing.

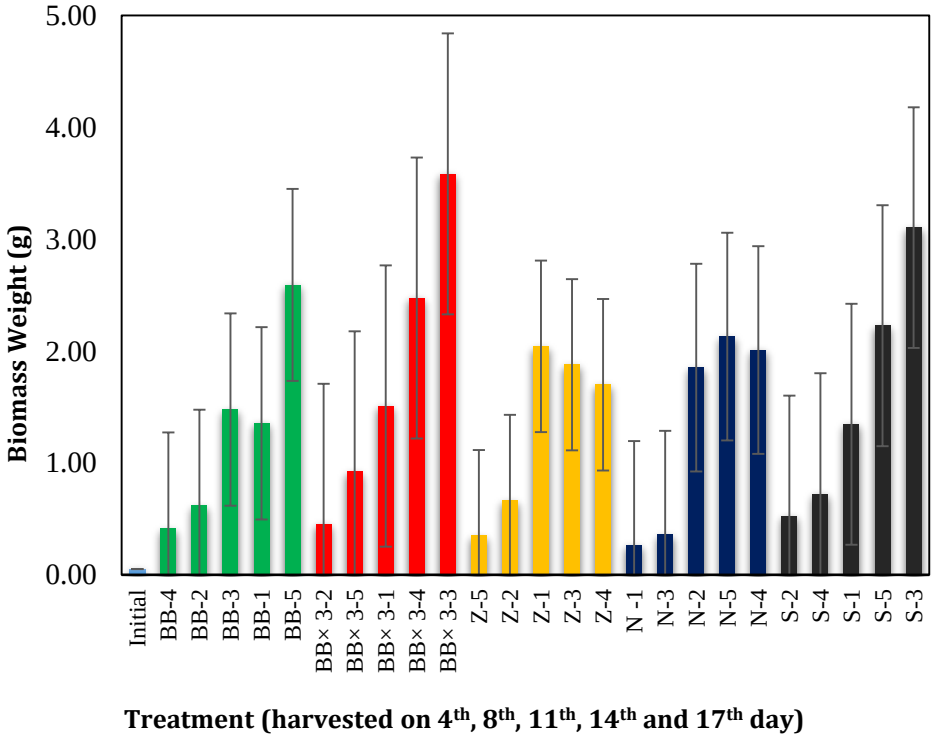

Figure 3: Dry biomass yield of different treatments at different harvesting days (4th, 8th, 11th, 14th and 17th day). The labels in the x-axis of the chart indicate the randomly collected pouch number of each treatment.

A gradual decrease of biomass was observed in Zinc stress treatment (Z) on 14th and 17thday compared to the 11th day of experiment due to lack of nutrients in the medium. The previous studies suggest that increasing Zinc concentrations can reduce the biomass production in microalgae *Chlorella* Sp. (Kondzior and Butarewicz, 2018). Therefore, the decrease of biomass did not seem to be induced by the lack of Zinc in the treatment. The peak value for the harvested biomass under zinc stress has been identified on the 11th day of the experiment.

In the nitrogen stress treatment, the highest biomass weight was obtained on the 14th day of the experiment which was slightly higher than the peak of zinc stressed treatments. However, it was still lower than the other 3 treatments namely; BB, BBx3, S (Salinity stress). The salinity stressed treatment showed a continuous increase in the biomass production with the increased salinity. Therefore, it shows the *Chlorella* Sp. mixed culture is well tolerant against the salinity stress. The highest average biomass concentration of 0.715 g/L with a standard deviation of ±0.349 was obtained in BBx3 treatment as it contained three times nutrients compared to normal Bold's basal media.

Variation of lipid percentage according to the treatment and harvesting date

A well distinguished difference was observed in the lipid production of Zinc stressed treatments from the first day to final day of the biomass harvesting (Figure 3). The least lipid percentage was observed in zinc stress treatment (4th day) which was higher than the peak lipid percentage obtained in BBx3 treatments. The highest lipid percentage of all treatments was obtained on the 14th day of experiments with Zinc stress medium whereas the lowest was recorded on the 8th day of experiment with BB medium. The percentage of lipid was ranging around 10% throughout all harvesting days in the BBx3 treatment. The lipid percentage of both nitrogen stress and salinity stress treatments were consistently increasing till the 14thday of experiment and showed a significant drop on the final day. This is due to the cell disruption triggered by lack of nutrients. The results of the previous studies also confirmed the primitive role of salinity stress in triggering the lipid production in microalgae species. Microalgae accumulate lipids according to the growth conditions and environmental stress (Gour *et al*., 2019)

As shown in Figure 4, the decline of nitrogen in media reduces the biomass production after 14 days. But it is still a substantial yield due to addition of nutrients on the 8th day of the experiment. The lipid production rate of the nitrogen stress media increases with the continued starvation that agrees with the finding of literature where they state nitrogen starvation could induce neutral lipid accumulation in *Chlorella* Sp*.* (Zhu *et al*., 2016). Microalgae use as biodiesel feedstock should ideally show high biomass productivity and efficient biosynthesis of lipids, i.e., it is not the single parameter (lipid content or growth

rate) but the volumetric lipid productivity that should be the main criterion for choice of feedstock for biodiesel production (Zhu *et al.*, 2016

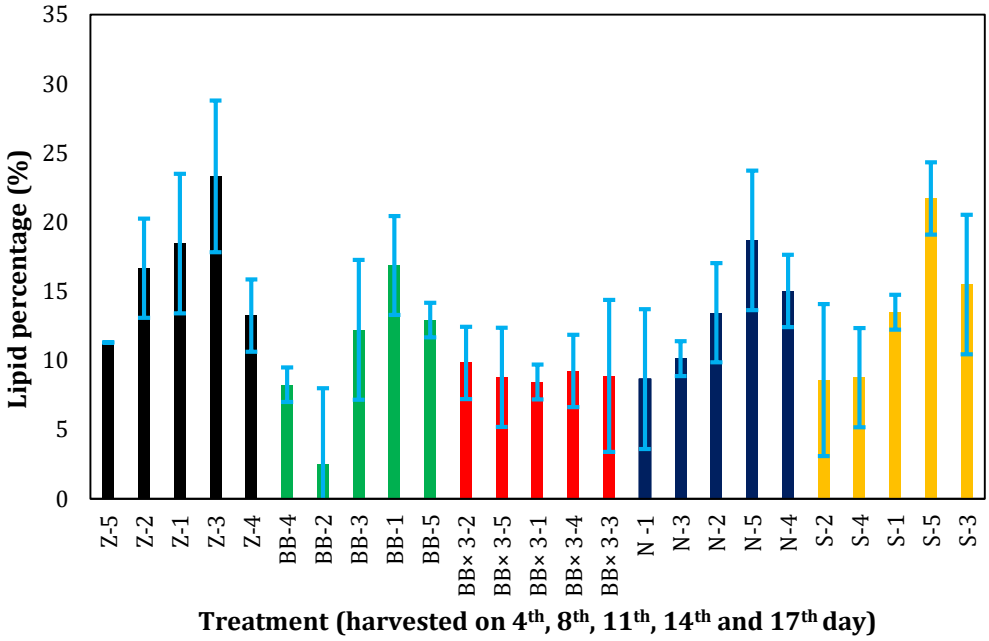

Figure 4: Lipid percentage in the treatments on different harvesting days. (Labels on the bars indicate the randomly collected pouch number in each treatment. Replicates from left to right are 4th, 8th, 11th, 14th and 17th harvesting days respectively.)

Generally, all the kinds of lipids in microalgae cannot be converted into fatty acid methyl esters (FAME), for example, fatty acids without O-linkage won't be converted into FAME. All types of fatty acids with *O*-ester linkages and Free Fatty Acids (FFA) can be converted almost quantitatively into the corresponding methyl esters in one-step reactions (Ichihara and Fukubayashi, 2010). Therefore, the conversion of microalgae lipids into FAME is a measure of microalgae lipids that are convertible into FAMEs. The available fatty acids are an important factor in biofuel production as well as in promoting value added products in industries such as food and pharmaceuticals. The fatty acids which were present in the 05 different treatments are listed below in Table 2.

Table 3: Major fatty acids and their percentages in different treatments obtained by FAME analysis

Name of the Fatty Acid	Major fatty acid Percentages					Standard Deviation
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	
Caproic acid (C6:0)	6.76	6.97	2.55	0.64	1.32	±3.02
Undecanoic acid (C11:0)	3.51	3.42	5.28	4.01	0.00	±1.96
Oleic acid (C18:1n9c)	38.32	36.20	39.87	45.38	43.83	±3.82
Linoleic acid (C18:2n6t)	20.24	2.50	34.52	22.05	4.21	±13.38
cis-11-Eicosenoic acid (C20:1)	0.00	7.70	0.00	0.00	0.00	±3.44
Heneicosanoic acid (C21:0)	0.75	22.86	6.77	11.05	22.67	±9.79
cis-11,14-Eicosadienoic acid (C20:2)	7.55	5.99	2.09	2.86	12.37	±4.12
Cis-8,11,14-Eicosatrienoic acid (C20:3n6)	6.64	0.00	0.00	0.00	0.00	±2.97
Arachidonic acid (C20:4n6)	7.30	1.79	1.35	1.64	3.99	±2.86

A total of 22 fatty acids have been identified and 8 of them were present in all the treatments at least in a minute percentage. Oleic acids have recorded the highest percentage in all the treatments where it hits the peak in nitrogen stressed treatment (45.38%). According to the literature, the Oleic acid is present in a variety of food resources around a percentage of 50% of the total fatty acid concentration and hasn't found any significant level of toxicity (Acid *et al*., 1987). Moreover, in a previous paper (Shim *et al*., 2018), the authors state it can be used as a biofuel by following deoxygenation, and the biofuel produced by Oleic acid using the CoMo-SG catalyst exhibited

a calorific value (10,119 cal/g) that was similar to that of commercial diesel (10,180 cal/g). The percentage of Oleic acid (45.38%) in this study is comparatively higher than that reported by Zhu *et al*. (2015) where they obtained 12 – 35% of lipids under Nitrogen stress with *Chlorella zofingiensis*. Oleic acid is considered ideal for biodiesel because it has better cold flow properties without losses to oxidative degradation. (Zhu *et al*., 2015). The Linoleic acid showed significant percentages over 20% in 03 treatments whereas the highest percentage of 34.52% was observed in Zinc stress treatment. Earlier studies revealed that linoleic acid is the highly consumed poly unsaturated fatty acid in human diet (Whelan and Fritsche, 2013) and it is also convertible into ethanol thus into biofuel. Because of the higher percentages of significant fatty acids, the stressing conditions can be helpful in producing biofuel efficiently.

Biomass productivity and lipid productivity

The highest average biomass productivity 0.060±0.017 $gL^{-1}d^{-1}$ was observed in BBx3 treatment where it had an extra amount of nutrients compared to other four treatments which were responsible for biomass production (Figure 5). The lowest biomass productivity 0.044±0.021 $gL^{-1}d^{-1}$ was recorded in Nitrogen stress media because the nutrients such as N, P, K play a major role in producing the biomass in microalgae (Mahmood and Khudhair, 2017). The coefficient of variation for the average biomass productivity values for BB, BBx3, Zn stress, Nitrogen stress and Salinity stress are 3.75, 3.57, 2.76, 2.06, and 3.90 respectively, which are on the lower side meaning that the results show more consistency.

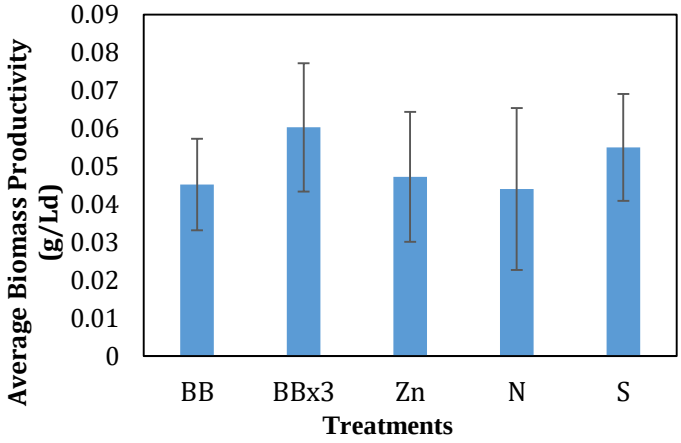

Figure 5: Average biomass productivity of Chlorella sp. microalgae in different treatments

As shown in Figure 6, the highest average lipid productivity 0.008±0.005 $mgL^{-1}d^{-1}$ was achieved in Zn stress treatment because of the high stressful conditions which force microalgae to store their food in the form of lipids to sustain in unfavourable conditions. Although, BB and BBx3 treatments recorded almost similar results 0.005±0.003 $mgL^{-1}d^{-1}$ and 0.005±0.110 $mgL^{-1}d^{-1}$ respectively suggesting the favourable conditions produce less amount of lipids compared to extreme conditions. An important observation in the present study is that the lipid content and the lipid productivity cannot be achieved in a same treatment. Thus, our study provides considerable insights into the enhancement of lipid content with respect to stress period.

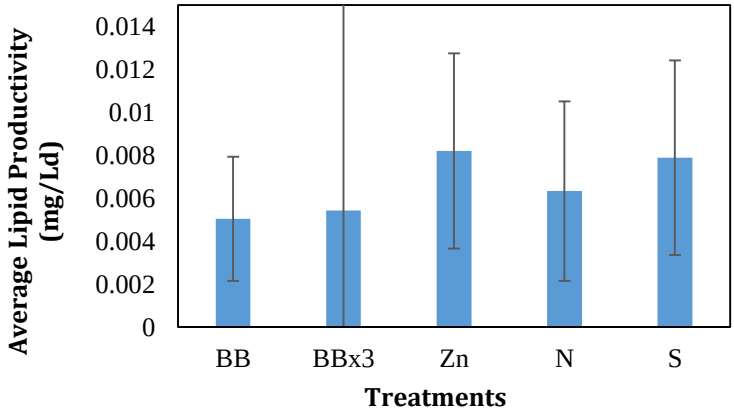

Figure 6: Average biomass productivity of Chlorella sp. microalgae in different treatments

Conclusion

The present study confirmed that the lipids in *Chlorella* Sp. can be enhanced by introducing various stress conditions in the culture media. It was clearly demonstrated that the biomass productivity and lipid productivity can be achieved in two consecutive phases in the same bioreactor configuration. Enhancement of biomass productivity required optimum addition of nutrients. Whereas, stressful conditions were required to enhance the lipid content and lipid productivity, Zn-stressed biomass exhibited the highest lipid percentage of 23.3% and the highest average lipid productivity of 0.008±0.005 mg lipids $L^{-1}d^{-1}$. The extracted lipids from the biomass yield contained important fatty acids such as Oleic acid and Linoleic acid in all treatments. All the treatments confirmed a substantial amount of biomass yield and lipid yield around 14^{th} day of the experiment. Therefore, it can be suggested to perform the cultivation in stressful conditions for 14 days to get maximum efficiency.

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Optimizing pressurized agriculture pipe network through simulation modeling techniques in home gardening in Vavuniya area of Sri Lanka

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Abstract

Home gardening is identified as one of the remedial measures for food security issues caused by the economic downfall in Sri Lanka. Home gardening contributes to household food security by providing direct access to food. Further, it is also an important source of supplementary income for households. The appropriate function of a pressurized agriculture pipe network system is vital to supply sufficient quantity of water to the plants at sufficient pressure through the sprinkler output. Despite this, very few computer simulation techniques studied the optimization for the case of the home gardening agricultural pipe system. In this study, the WaterGEMS V8i software simulator was used and hydraulic analyses were conducted to design an optimal pressurized agriculture pipe network suitable for home gardening in the Vavuniya area. The water flow rate at sprinkler outlet points for chilli and onion were considered 600 L/h and 325 L/h respectively.

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The total available land area for home gardening was considered as 50 perches in this study. Fifteen models were developed for different combinations of land use for chilli and onion cultivation. The hydraulic parameters such as nodal pressure, flow velocity, flow rate and head requirement were analyzed under steady-state simulation by using the Hazen-Williams friction method. The proposed simulation model was calibrated and validated by using a previous study. The result revealed that all of the nodes in the system are operating above the threshold pressure limit of 2 bars. It was found that a combination of 20% land (10 perches) for onion and 80% land (40 perches) for chilli required the highest power pump capacity of 3.5 kW. The power of the water pump for chilli only and onion only was positively correlated with the cultivated land extent and R^2 values were observed to be 0.9991, and 1.0 for chilli and onion respectively. The flow velocity in all pipes was above the minimum level of 0.5 m/s eliminating silt deposition and below the maximum level of 2.0 m/s avoiding the water hammer issues. The proposed pressurized agriculture pipe network design shall be used for modelling of pipe network for home gardening with different crop types by changing the model input parameters by using the WaterGEMS V8i computer simulator.

Keywords: Home gardening; Optimal design; Sprinkler irrigation; WaterGEMS V8i; Water hammer

Introduction

The Earth's surface is surrounded by water by seventy-one percent, however, approximately only three percent is freshwater. Hence, freshwater is categorized as a limited and vulnerable resource. Water supply to agriculture plays a vital role in the productivity efficiency. The conventional canal system is used to supply water from water sources. However, science is advanced and contributed to inventing the agricultural techniques like lift irrigation, sprinkler, mechanical device, which created rapid developments.

Sri Lanka is primarily an agriculture-based country. Agriculture has contributed to Sri Lanka's GDP by 7.8 per cent in the first quarter of 2021. Home gardening is identified as one of the remedial measures for food security issues caused by the economic downfall in Sri Lanka. Home gardening contributes to access to food and a source income for households.

Gravitational force is used to irrigate the irrigable areas which are topographically situated below the water level. Furthermore, this kind of gravity-based irrigation is a convenient method for the reason that it requires less workforce and low cost. But in the case of a region which is located above the water level, gravitational force cannot be used to carry water to that region or land. Lift irrigation is the solely way to supply water to such regions under irrigation. In lift irrigation, pumps are utilized to lift water from the neighboring water source and followed by distribution to the crop (Shiyekar & Patil, 2017)

A pressurized agriculture pipe network shall be hydraulically analyzed through computerized simulation techniques and simulated for the different scenarios to optimize the pipe diameters and pump capacity. The appropriate function of a pressurized agriculture pipe network system is vital to supply sufficient quantity to crops, gardens, vegetables, etc.at sufficient pressure in the sprinkler output. Hence, the principles of designing such a pressurized system need to be well understood.

The present study aims to model, simulate, and design an optimized pressurized agriculture pipe network system by maintaining adequate flow and pressure head at sprinkler outlet for home gardening suitable for the Vavuniya area of Sri Lanka.

Study area

The area for modelling of optimized pressurized agriculture pipe network suitable for home gardening is the Vavuniya area in the Northern part of Sri Lanka as shown in Figure 1(a). As per the "land use plan" in the Vavuniya district (Ministry of Lands, 2016), the land extent of 12,841.6 ha is identified as an underutilized home gardens area as shown by yellow patches in Figure 1 (b). One of the major reasons is inadequate technical know-how and poor water management.

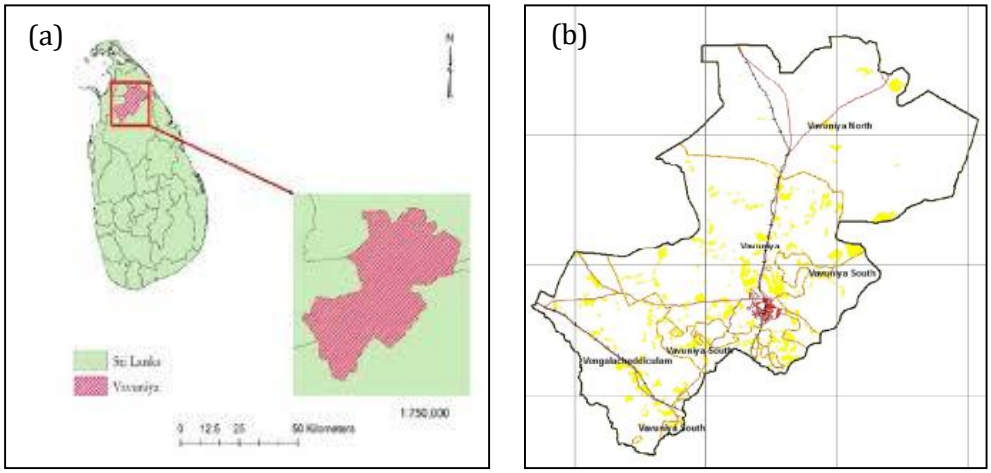

Figure 1: Study area (a); Area with high proportion of underutilized home gardens in Vavuniya (b)

Pressurized agriculture pipe network system

A pressurized pipe network system is a combination of pipes, valves, fittings, water meters, pumps or elevated water tanks, which are analyzed, designed, optimized and installed. A properly designed and installed pressurized agriculture pipe network system can reduce the frequency of maintenance, inspection, and rehabilitation during the operation of the network system. Therefore, analysis and design of the system shall adversely affect the efficiency of any agriculture. A study was done in sprinkler irrigation system in Iraq and revealed the ability of applying these models on other regions other Iraq governorates (Al-adili & Kraidi, 2015). A program namely; EPANET was used to model the pressurized drip irrigation system to understand how the piped irrigation system operated (Sharu & Razak, 2020).

Need for home gardening

The country's economic situation and COVID-19 pandemic brought significant impacts on the society and the threat to immediate food security are on top of the list. Home gardening is identified as one of the remedial measures for this situation. The interest of the society in home gardening has drastically increased especially midst of the pandemic. On the other hand, improper water management for home gardening shall contribute to t increase the domestic per capita water usage in the country.

The district development plan for 2018-2022 of Vavuniya district indicates the annual target of land extent for cultivating the cash crops such as chilli and onion as plotted in Figure 2, by taking the baseline cultivated area as 300 ha and 66 ha for chilli and onion respectively (District Secretariat, 2017).

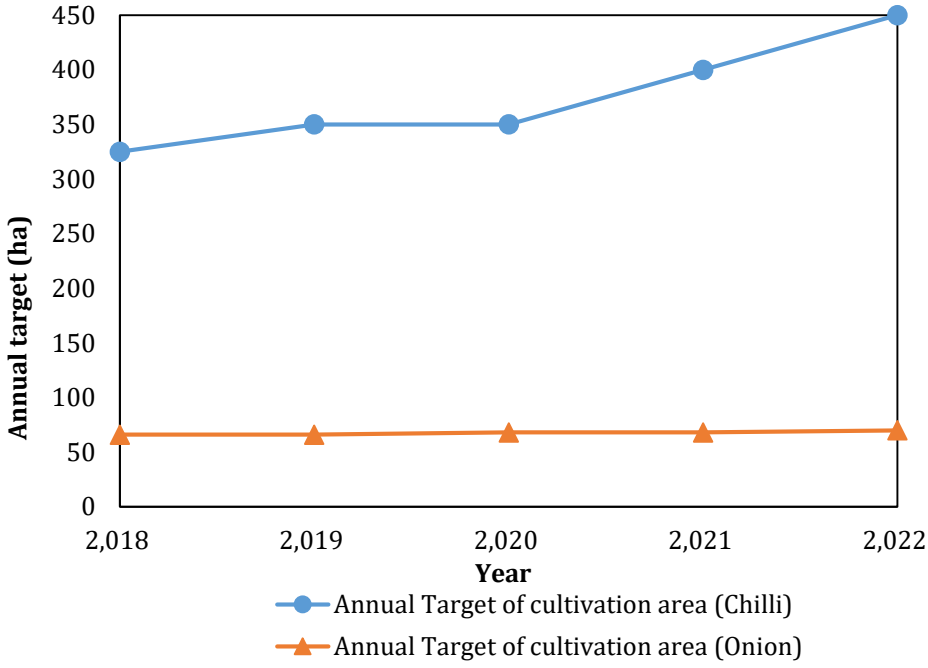

Figure 2: Annual targets of cultivating land extent for chilli and onion in Vavuniya

Need for pipe network design and optimization

Water is one of the most vulnerable and finite resources on earth. Water plays a vital role in the economic development of any nation, fulfilling the human's basic needs, agriculture developments, etc. Climate change is worsening water scarcity, which is a nationwide, within the nation, seasonally-related issue. Hence, using water sparingly for agricultural purposes is inevitable in the current context.

Saving water has become a critical problem particularly when a huge amount of water is being used for the activities such as irrigation and agriculture. Home gardening is a water-consuming process which consumes a considerably higher amount of water than water required for other human activities at the

household level. Plants and crops absorb a definite amount of water and the balance supplied water may be wasted via seepage, runoff, evaporations and any other means. Therefore, the supply of water to the crops especially in home gardening is to be dealt with additional care to ensure springy water usage. Properly designed and optimized pressurized agriculture pipe network system for home gardening shall not only supply an adequate quantity of water to the crops and reduce the capital investment cost but also curtail unnecessary wastage of water, by doing so, additional cultivable command area or irrigable area shall be irrigated which will generate additional income to the household and will contribute to achieving the self-sufficiency on food in the country.

Materials and methods

Methods of model preparation and analysis

The peak water flow rate at sprinkler outlet points for cash crops such as chilli and onion was taken into consideration as 600l/h and 325 l/h respectively as per the drip and sprinkler irrigation manual of the Department of Agriculture. The rotary type sprinkler, which is capable to cater the water requirement of the land extent of 5m x 5m, was used in the model. The total available land for home gardening considered in this study was 50 perches, 15 nos of models developed for a different combination of land use for chilli and onion cultivation. Ten models of pipe networks were created for different land used areas as 10, 20, 30 40 and 50 perches for the cultivation of chilli and onion crops each (each crop has 05 models). Balance 05 models were developed for the land use pattern of combination of chilli and onion crop (Onion 0% and Chilli 100%; Onion 20% and Chilli 80%; Onion 40% and Chilli 60%; Onion 60% and Chilli 40%; Onion 80% and Chilli 20%; and Onion 100% and Chilli 0%).

WaterGEMS V8i Simulator

A user-friendly interface to analyze, design and optimize WDN is provided by WaterGEMS V8i software. There are a few important attributes namely hydraulic analysis, water quality analysis, extended period simulation, and steady state simulation. Simplified model building, water quality modelling, fire flow analysis, optimization and scenario management are some benefits of WaterGEMS V8i over other software (Sonaje & Joshi, 2015). Further, it is

recorded that, application of using the HydroCalc program can be also used to choose the appropriate design for the irrigation system (Darwish et al., 2022)

Pipe network modeling procedure

The computer simulator software namely WaterGEMS v8i was used for analysis and design. The following steps were carried out to create the computer model of the pipe network using WaterGEMS simulator.

Step 01 – Input data

The input required data for analysis is the first step in any hydraulic analysis programme especially in WaterGEMS. The input data are categorized into four types namely pump data, tank data, junction data and pipe data. However, the tank data part is not included in the methodology, since the system is fed by pumping system

The most important data such as pump number, capacity (kW), base elevation (m), and pump flow rate (l/s) were input under tank input data. Pipe diameter (mm), pipe material, pipe number, dimensionless roughness coefficient, pipe length (m) and minor losses were assigned as pipe data. Under junction data, the important data such as junction number, elevation (m), junction demand (l/s), and demand pattern were assigned.

Step 02 –Network simulation

This is a vital part of the hydraulic analysis in WaterGEMS software. The model was checked for errors after creating the model and input data through a process called the "validate" option. Then the model was simulated with a hydraulic run after inputting all the required data as shown in Figure 3. Nodal pressure (m), flow velocity (m/s), pipe head loss (m) and head loss gradient (m/km) were computed by the software which is vital for decision-making during hydraulic analysis. As per Bentley (2013), waterGEMS V8i programme has a reliable design algorithm to produce precise network design since the WDN parameters namely junction pressure, velocity and water flow along with their optimization are controlled by the programme.

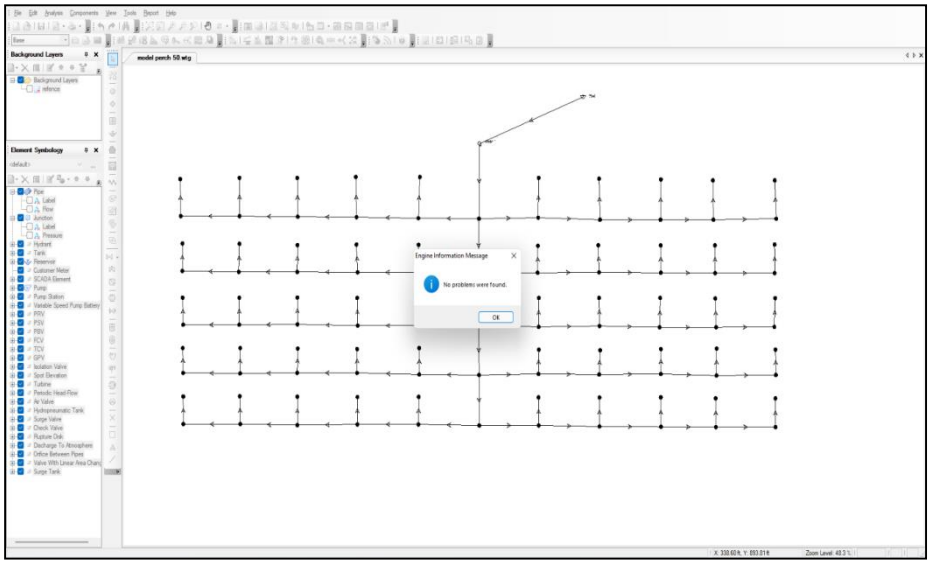

Figure 3: Model validation process

Step 03 –Hydraulic run

The hydraulic run was performed as shown in Figure 4 and the required hydraulic output parameters were extracted from the report section of the software. The important output parameters such as pressure at every junction and the flow velocity in every pipe were checked to have them within the acceptable range.

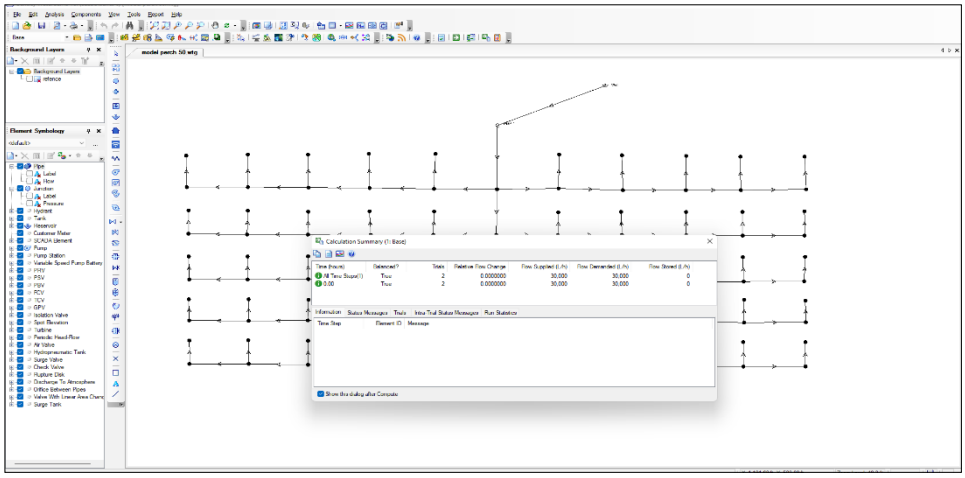

Figure 4: Hydraulic run

Step 04 – Finalization of network configuration

The model was simulated repetitively for several trials by adjusting the data until a satisfactory water network configuration is reached. The network model was created with the relevant elements such as pipes, nodes, bends, valves, and pumps as illustrated in Figure 5.

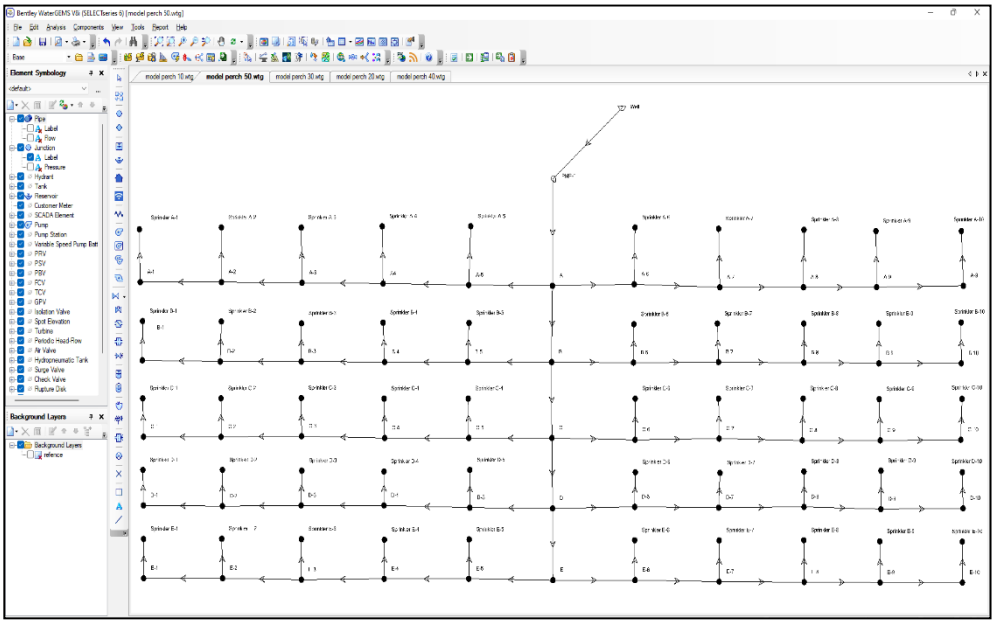

Figure 5: Agriculture pipe network computer model

Step 05 – Result and analysis

The model was run for the last time once the water network configuration is accepted. The required tables and graphs were extracted from the generated results.

This is a simple system fed by a pump which was designed to have sufficient head and water flow. Next, pipe data were created with the following; pipe length, pipe material, internal diameter, and Hazen-Williams friction coefficient. The node data were input with demand and elevation above the given datum. The hydraulic analysis is carried out by using the Hazen-Williams friction method (Equation 1) with steady state simulation in this study.

$$\frac{H_L}{L} = \frac{10.67\ Q^{1.852}}{C^{1.852}\ D^{4.8704}} \qquad \text{Equation 3}$$

Where;

Q = Fluid flow rate (m^3 /s)

C = Dimensionless HW roughness constant

D = Pipe internal diameter (m)

H_L = Head loss (m)

L = Pipe length (m)

Model validation

The model validation is a vital part in any research, in order to confirm the capability of software used for modeling and to confirm whether the researcher has enough knowledge on the modeling. The proposed simulation model was validated and calibrated by using the study done by Revelli & Ridolfi (2002) before the analysis. The results of flow measurement in every pipe were used for validation. The deviation between the flow results of this study model and the study of Revelli & Ridolfi (2002) is tabulated in Table 1, with an average deviation of 1.98%.

Table 1: Summary of model validation

Start Node	*Stop Node*	*Flow (m^3/s) [current study]*	*Flow (m^3/s) [previous study]*	*Results Deviation (%)*
K1	k2	0.452	0.458	1.3%
K2	K3	0.221	0.224	1.4%
K4	K3	0.078	0.076	2.6%
K1	K4	0.227	0.222	2.2%
k2	K4	0.081	0.083	2.5%

Results and discussion

There are few significant output parameters required to analyze the optimizing options to design the agriculture pipe network, in terms of investment cost, operational efficiency and operation cost. Those output parameters mainly nodal pressure, flow rate, flow velocity, and capacity of the pump were extracted from Water GEMS simulation models and discussed in this chapter.

The capacity of the pumps

The models were simulated several times to optimize the pressure in all the nodes (sprinkler points) and the flow velocity in all the pipes for different combinations. Figure 6 illustrates the pump capacity requirement for (a) different combinations of chilli and onion, (b) different land used areas for chilli only and (c) different land used areas for onion only. It can be observed that 20% land (10 perches) for onion and 80% land (40 perches) for chilli required a higher power water pump. The power of the water pump for chilli only and onion only is positively correlated with the cultivated land extent and R^2 values were observed to be 0.9991, and 1.0 for chilli and onion respectively.

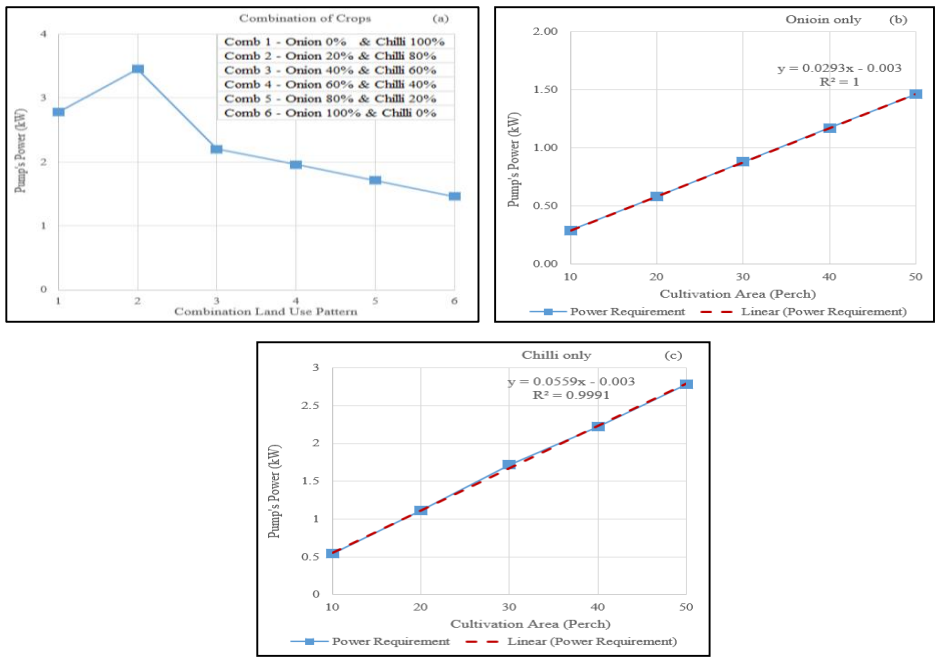

Figure 6: Power requirement for different combinations of cultivation (a); combination of crops Onion only (b); Chilli only (c)

Flow velocity

The minimum flow velocity in WDN is specified as 0.5 meters per second to eliminate the chance of slit deposition which may occur due to the low velocity as specified in the design manual of the water network(National Water Supply & Drainage Board, 1989). As Figure 7 shows, the flow velocity in all pipes was observed to be above the minimum level. The maximum velocity level is specified as 2.0 m/s to eliminate the water hammer scenario, which can cause failure in the pipe, however, the velocity is well within the maximum level in the system.

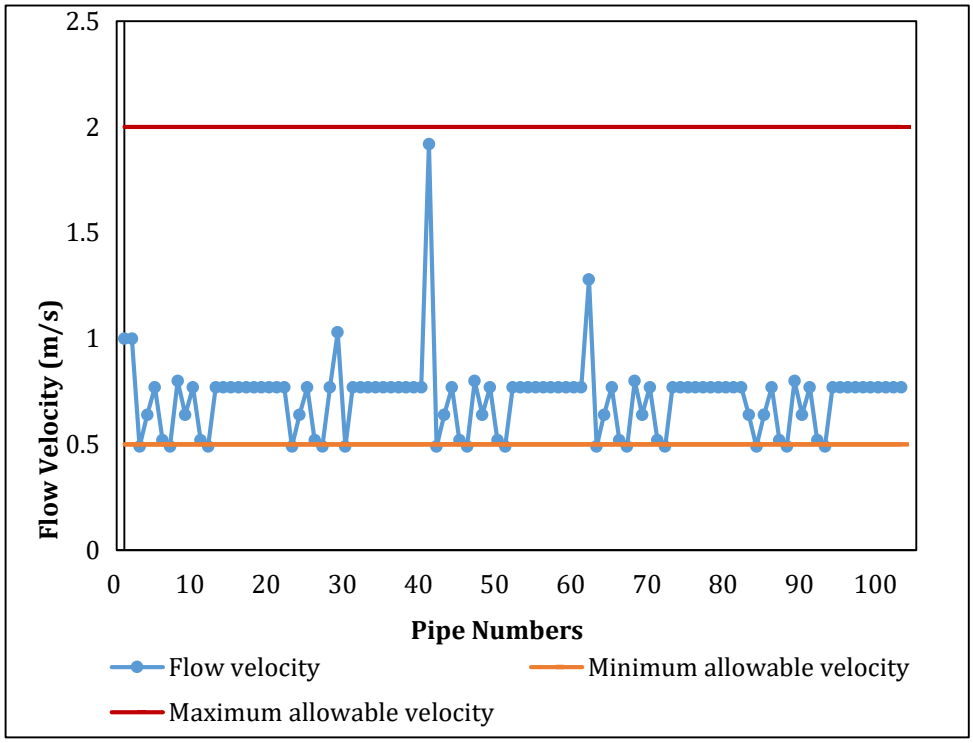

Figure 7: Flow velocity in the pipes

Pressure at sprinkler point

The pressure at the end point of each sprinkler is plotted in Figure 8.The pressure is one of the critical output factors which shall give an idea of ensuring

the supply of water through every sprinkler for the efficient watering of the plants. The pressure in the pressurized agriculture pipe network mainly depends on pipe diameter, roughness coefficient of pipe material, and elevation (Liang et al., 2004). As mentioned in the drip and sprinkler irrigation manual of the Department of Agriculture, the minimum pressure is to be maintained above 2 bars at the sprinkler when using rotary type sprinkler. As such the result indicates that the nodal pressure in this particular pipe network is above the minimum level and adequate for the effective performance of the agriculture pipe network designed for home gardening.

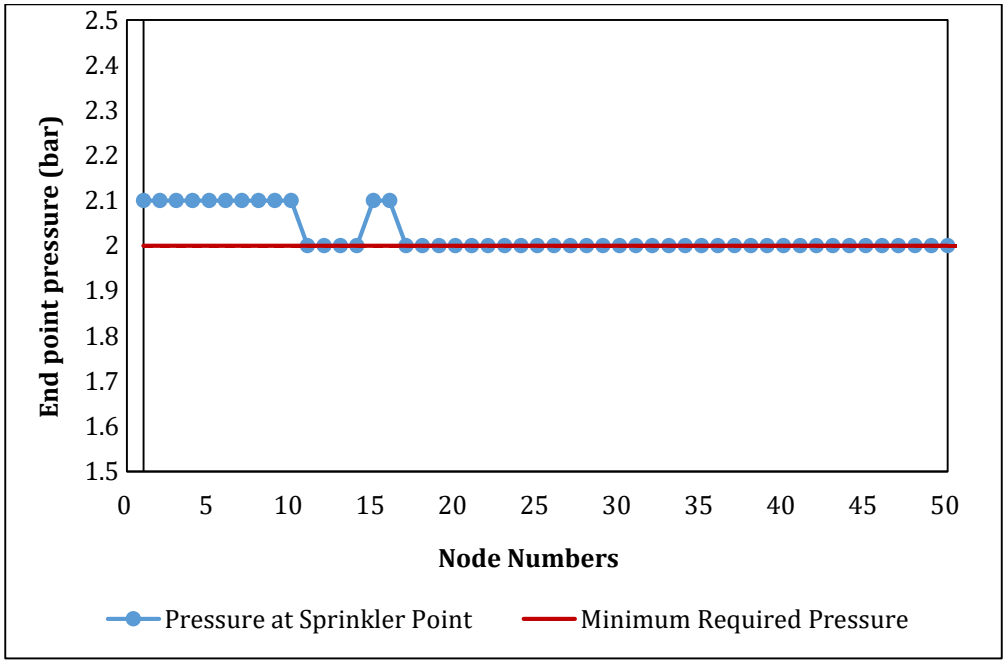

Figure 8: Pressure at sprinkler point

Conclusion

Based on this study, the vital output parameters such as the flow velocity in all the pipes and nodal pressure at all the nodes are sufficient enough to supply water as per crop water demand in the study area. The designed and optimized pipe sizes of the pressurized agriculture pipe network are adequate to meet the water demand while maintaining adequate pressure in the system. A network designed using WaterGEMS satisfied the design criteria considered for

pressurized pipe networks. The optimal design of the pipe network was carried out to satisfy the positive pressures at all the nodes which indicate an assured supply of water up to the tail end sprinkler. The proposed pressurized agriculture pipe network design shall significantly reduce the seepage losses if implemented in practice for home gardening. The results suggest that WaterGEMS is a reliable simulating tool for developing drip irrigation systems especially suitable for home gardening in selecting the economic pipe diameter, reducing the capital cost of establishing an agricultural water network, and achieving the optimal pumping operating pressure. The WaterGEMS simulators used for this design can handle various pressurized agriculture pipe network problems. Computer-aided network simulation techniques provide great advantages over conservational computations in terms of optimization, results in accuracy, monitoring of the system during operation, time consumption and room for future modification. The proposed pressurized agriculture pipe network design shall be used for modeling of pipe network for home gardening with different crop types by changing the model input parameters.

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