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PREFACE

ICA Research Symposium (ICARS) is an annual symposium organised by Innovation Centre in Agritechnology for Advanced Bioprocessing (ICA), Universiti Teknologi Malaysia, to provide a platform for researchers and industrial practitioners to share their research findings, exchange ideas, innovation, knowledge and experiences which eventually lead to great networking and collaboration opportunities.

The 3rd ICA Research Symposium (ICARS) 2020 was held for the first time through an online platform on 22nd September 2020. A total of 21 participants from various higher education institutions were selected to present their recent research findings and inventions in this one-day symposium. 3rd ICARS 2020 focused on sustainable agriculture, food & nutrition security.

This proceeding contains 13 selected extended abstracts representing main topics in 3rd ICARS 2020. The Editors wish to thank all the authors who have contributed to this proceeding. We also acknowledge all our reviewers for contributing their valuable time and expertise to ensure an efficient peer-review process and maintain the high standard of ICARS proceedings.

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Composition study of leaching from monoculture and polyculture of water spinach, okra and yard long bean

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Abstract
Intensive use of synthetic fertilizers often leads to agriculture surface runoff which pollutes the water system. In response, various best management practices (BMPs) have been established with the objective to reduce the use of synthetic fertilizers and their adverse effects to environment. This study looked into both monoculture and polyculture comparatively. Water spinach, okra and yard long bean were grown under both monoculture and polyculture system. The experiment was in completely randomized block design with three replications per cropping system. Each cropping system were fertilized using organic fertilizer (NPK 3:3:3) with application of 9 g/m^2 twice a week until harvesting. Soil water sampler was installed under the planting bed at the depth of 30 cm. Soil water sample were collected at the end of experiment. Leachate samples were analyzed. The data for pH, EC, ammonia, nitrite, nitrate, chloride, hardness, total dissolved solid were recorded and analyzed with One-way ANOVA ($p<0.05$). There was no significant difference ($p>0.05$) between monoculture and polyculture crop using organic planting method. Nitrate level showed different value in monoculture water spinach, monoculture yard long bean, monoculture okra and polyculture crop (0.9533 ppm, 0.8067 ppm, 0.0000 ppm, 1.100 ppm). Accurate fertilization increases nitrogen use efficiency and reduces the risk of leaching during the plant growth. In conclusion, under organically managed soil the leachate of polyculture crop system has similar leachate composition to monoculture cropping system.

Keywords: Leachate, monoculture crop, polyculture crop, organic fertilizer

1. Introduction

Leaching from agriculture activities is a primary source of dissolved inorganic nitrogen (NO_3^-, NO_2^-, NH_3) which affects surface and ground water (i.e: eutrophication and acidification). Improper management of agricultural land lead to soil fertility problem and soil degradation such as landslides, soil erosion and nutrient leaching. Synthetic fertilizer drives the potential of nutrient leaching become one of the factors contributes to the deterioration and water pollution [1]. Effluent with heavily loaded nutrient from agriculture activities is unsuitable for human and animal consumption and also direct release to environment. Eventually, nutrient released by agriculture activities beyond the treshold level not suitable for farming. In Malaysia, Department of Environment (DOE) had developed National Water Quality Standard for Malaysia to be a guideline for agriculture been released to the environment [2]. Best practices of nutrient management

is important to reduce the leaching contamination to environment. Improved root architecture systems design can help crops utilize soil resources more effectively. Nitrate is the available form of nitrogen which is soluble in water. Surplus of nitrate in the soil that unable to absorb by the roots will cause nitrogen leaching downward the root zone [3]. Nitrogen loss rely on the amount of accessibility water passing through the bottom of soil under the root zone. High precipitation, rainfall or humid area also one of the factors in the occurrence of leaching. [4]. Monoculture system is cultivating single crop species using the majority or whole of the land during growing season. Polyculture system is cultivating two or more crop species simultaneously in the same field during the same growing season [5]. Polyculture system allowed various roots length development in soil and it is believed to enhance soil stability. This cropping system have been accepted as sustainable farming practices [6]. Nitrification process is the conversion of ammonia to nitrate which will be absorbed by the plant as nutrient. Objective of this study is to evaluate the effect of different organically managed cropping system on water leaching quality.

2. Materials and methods

Okra, water spinach and yard long bean were grown under both monoculture and polyculture systems. The experiment was conducted in completely randomized design with three replications per cropping system. Each cropping system were fertilized using organic fertilizer (NPK 3:3:3) with application rate of 9 g/m^2 twice a week until harvesting. Soil water samplers were installed under each planting bed at the depth of 30 cm. Soil water sample were collected at the end of experiment. Leachate sample were analyzed following method of state by American Health Public Association (APHA) [7]. The data for pH, electroconductivity (EC), ammonia, nitrite, nitrate, chloride, hardness, total dissolved solid (TDS) were recorded and analyzed with One-way ANOVA ($p<0.05$).

3. Results and discussion

Table 1: Physiochemical characteristics of leachate from different cropping system

Parameters	Monoculture			Polyculture
	Water spinach	Yard long bean	Okra	
pH	6.3 ± 0.9[a]	6.2 ±0.8[a]	6.9 ± 0.1[a]	7.1 ± 0.2[a]
EC (µS/cm)	673.70 ±119.70[a]	456.13 ±162.08 [a]	513.17 ±96.06[a]	1218.40 ± 470.67 [a]
Ammonia (mg/L)	0.00 ± 0.00[a]	8.80 ±7.63[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Nitrite (mg/L)	10.00 ±2.50[a]	2.50 ±2.50[a]	5.00 ± 5.00[a]	5.00 ±2.50 [a]
Nitrate (mg/L)	0.95 ± 0.95[a]	0.81 ±0.81[a]	0.00 ±0.00[a]	1.10 ± 0.63[a]
Chloride (mg/L)	33.33 ±13.33[a]	26.67 ±3.33[a]	36.67 ±3.33[a]	50.00 ± 11.55[a]
Hardness (mg/L)	52.00 ±15.72[a]	37.00 ±4.36[a]	45.00 ±21.28[a]	59.00 ± 15.72[a]

Parameters	Monoculture			Polyculture
	Water spinach	Yard long bean	Okra	
TDS (mg/L)	523.33 ±262.64[a]	183.33 ±18.56[a]	116.00 ±21.57[a]	146.00 ±15.54[a]

Mean ± SE followed by different letters in the same row are statistically different according to Duncan's multiple range test p<0.05

The physiochemical quality of the leachate from different cropping system is reported in Table 1. There is no significant different (p>0.05) all the treatment. During harvesting day, the pH value is ranged from 6.2 ± 0.8 (monoculture yard long bean) to 7.1 ± 0.2 (polyculture). The pH level remained within safe range of 5-9 as set by DOE for effluent discharge [2]. A average pH value of 6.9 to 7.3 was found in leachate from central Anatolian part of Turkey [8]. Dissolving of ammonia in water leachate influenced the level of pH due to ammonium ion and carbonic acid dissociates to produce hydrogen cation. Increasing value of hydrogen cation in water leachate lowered the pH value. [9-10]. From this study, EC values were in the range of 456.13 ± 162.08 µS/cm to 673.70 ± 119.70 µS/cm for monoculture cropping, respectively. While, EC value for polyculture cropping was 1218.40 ± 470.67 µS/cm. Polyculture cropping system has higher EC value compared to monoculture cropping system. EC is related to the salinity of the leachate. Polyculture cropping covered the soil bed better than monoculture which may reduce the evaporation of water in the soil. High water content in soil increased the rate of leaching salts out of the root zone [11]. EC value has negative effect on vegetative growth [12]. Value of EC for leachate from Nkolfoulou landfill, Cameroon is reported about 20,450 µS/cm [10]. TDS value from 116.00 ± 21.57 mg/L (monoculture okra) to 523.33 ± 262.64 mg/L (monoculture water spinach). With respect to agriculture and livestock uses, EC and TDS values meets specific standard set by regulatory authorities [2, 13-15] as in Table 2. Hardness value from leachate of monoculture and polyculture crooping system vary from 37.00 ±4.36 mg/L (monoculture yardlong bean) to 59.00 ± 15.72 mg/L (polyculture). The hardness value is suitable as compared to standard limit for aquaculrure and recreational uses, which is 250 mg/L as $CaCO_3$ [DOE]. In this study, concentration of major anion like nitrate and chloride are within DOE safe limit [2] as in Table 2. Nitrate (NO_3^-) value is range from 0.00 mg/L (monoculture okra) to 1.10 ±0.63 mg/L (polyculture). Concentration of nitrates vary from 0.49 to 62.20 mg/L in agriculture area of Ningxia, Northwest China [16]. Chloride (Cl^-) value is ranged within 26.667 ±3.333 mg/L (yard long bean) to 50.00 ± 11.55 mg/L (polyculture). Safe limit of chloride state by DOE is below 80 mg/L. Comparatively, concentration of Chloride (Cl^-) studied in Turkey has higher [8] range of 70.90 mg/L to 120.50 mg/L. Chloride is an essential micronutrient needed by all crops in small quantities. Higher amount of chloride caused surface salt formation and increase the alkalinity of the water [17]. Nitrates represent as key indicator for domestic and agriculture pollution. In leachate samples, it is formed primarily as a result of nitrification process from ammonium to nitrites and subsequently, to nitrates.This process with present of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria [9]. Application of excessive fertilizer and irrigation water to obtain high yield during crop production caused serious environmental problems. For instance, in some vegetable cultivation area in China, annual irrigation rate was as high as 1000 mm, and fertilizer N efficiency was only 18-33% of applied N taken up by the vegetables. [18]. In some vegetable cultivation regions in Malaysia, annual irrigation rate was as high as 5315

mm, and fertilizer was about 54-57% of applied N taken up by vegetables [19]. Several studies have shown leaching of nutrient accumulation occurred when manure application exceeded crop requirements. Hence, application manure should be at equivalent levels to the crop nutrient demand. The effluent of nutrients from agriculture site depends on management factors such as rate and time of application, on feed composition and on soil and climatic factors. Both monoculture and polyculture crops have no different under organic managed system. Organic- granule fertilizer applied to the crops has slow release nutrient which reduce the risk nutrient leaching to the environment

Table 2: Water guidelines for agricultural uses

Parameter	EU [11]	EPA [12]	WHO [13]	DOE [2]
pH	6.5-9.5	6.5-8.5	-	5-9
EC (µS/cm)	2500	-	-	6000
Ammonia (mg/L)	-	-	-	5
Nitrite (mg/L)	-	-	-	100
Nitrate (mg/L)	50	45	50	100
Chloride (mg/L)	250	250	250	80
Hardness (mg/L)	-	-	-	250
TDS (mg/L)	-	500	1000	4000

Note: EU: European Union; EPA: Environment Protection Agency, United State; WHO: World Health Organization, DOE: Department of Environment, Malaysia.

4. Conclusions

Groundwater and surface water pollution are major environmental concerned; therefore, leachate quality deserve a careful investigation and analysis to prevent further damage to the environment. Even the effluent from agriculture leachate met the effluent standard, leachate is still one of the major sources of mineral discharged to the environment. Accurate fertilization increases N use efficiency and reduces the risk of leaching during the growth period. Organic fertilizer applied to the cropping systems promised low risk of water pollution to environment. Based on the experiment, application of correct amount of organic fertilizer could be recommended to the farm management for monoculture and polyculture system.

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Efficacy of microalgae as a nutraceutical and sustainable food supplement

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Abstract
With the rising global population, achieving nutritional food for all is a matter of concern. Therefore, sources of nutrients having low cost and can easily and speedily produce copious amounts of nutritional value are required. Microalgae stands out for that purpose since it can produce nutrients as well as nutraceutical compounds. "*Nutraceuticals*" which are the blend of "nutritional" and "pharmaceutical" are basically the substances which supplements the diet nutritionally and help in the prevention/treatment of diseases. *Chlorella vulgaris* is a health food supplement while other extracts of *chlorella* are antioxidant, anti-tumour and have the potential to lower high blood pressure and high cholesterol. *Dunaliella* contains Beta carotene (effective carotenoid) which increases immunity and reduces the risk of liver lesion. *Haematococcus* is the natural factory of astaxanthin which is 1000 times more potent than vitamin E and helps to reduce triglycerides and fatty acids. Protein extraction from microalgae is beneficial in terms of both productivity and nutritional value. The yield of protein from microalgae is 4-15 tons/ha/yr compared to 1.1 tons/ha/yr, 1-2 tons/ha/yr and 0.6-1.2 tons/ha/year for wheat, pulse legumes and soybean, respectively. Microalgae (eukaryotic and prokaryotic) are capable of bio-converting CO_2 (using light) into microalgae biomass which in turn is the potential source of biofuels, nutritional supplements, and pharmaceuticals. This paper provides an insight about the key role played by microalgae in the production of food and nutraceutical compounds and subsequently its derivatives can assist in supplementing and safeguarding human wellbeing in a sustainable manner, showing its efficacy as a promising food for future.

Keywords: Microalgae, functional food, nutraceutical, sustainable

1. Introduction

World population is projected to reach 9.7 billion people by 2050 [1]. 1 out of 9 people in the world is suffering from hunger/malnutrition. Expanding food production and economic growth have often come at a heavy cost to the natural environment. The Earth can fulfil the demands of food security; however, the agricultural sector will need innovative technologies and research to retrieve its full capacity for the production. Therefore, easy to produce and cost-effective sources that can rapidly produce substantial amounts of nutritional value are needed [2]. Microalgae can become a future food supplement and nutraceutical compound because of its unique composition. Microalgae (eukaryotic and prokaryotic) are capable of bio-converting CO_2 (using light) into microalgae biomass which in turn is the potential source of biofuels, nutritional supplements, and pharmaceuticals. Microalgae are having many advantage like high biomass yields per unit area, and the ability to be grown on non-arable land or in the photobioreactors (closed or open), they can be grown in saline water and wastewater [3]. Microalgae are extremely diverse group with estimated number of species ranging from

200,000 to 800,000, out of which about 30,000 are archived [4]. For a healthy lifestyle, a balanced diet constituting of antioxidants, vitamins, PUFAs, etc are required.

Numerous species of microalgae are reported to be rich in proteins, lipids, carbohydrates and other bio-active compounds [5]. The protein yield from microalgae is reported at 4-15 tons/Ha/year compared to terrestrial crops production of 1.1 tons/Ha/year, 1-2 tons/Ha/year and 0.6-1.2 tons/Ha/year for wheat, pulse legumes and soybean respectively [6].

2. Application of microalgae in nutraceutical compounds

Nutraceutical compounds are the products which supplement the food as well as helps to treat or prevent many types of diseases/disorders and simultaneously provide strength to the immune system (Table 1). About 470 nutraceutical compounds are known and found their application in the nutraceutical industry [7]. Microalgae are the rich sources of vitamins (A, B1, B2, C and E) and minerals (calcium, iron and magnesium) [8]. The applications of various microalgae in nutraceutical compounds with their salient features and the companies producing them are incorporated in Table 1.

Table 1: Different microalgae with their nutraceutical applications

No	Microalgae species	Salient features	Nutraceutical applications	Companies	References
1	***Chlorella***	55-67% protein, 1-4% chlorophyll 9-18% dietary fibers Good producer of lutein	Helps to prevent/treat muscular degeneration anti-cataract used for the treatment of high blood pressure and cholesterol	Lucky Vitamin™ Prime Chlorella™ Distribution Inc. Sun Chlorella, Herb Mark	[9, 10]
2	***Dunaliella***	*Dunaliella* produces copious quantity of beta carotene, Possess Antioxidant activity	Helps in the prevention of cancer various organs like lungs, stomach, cervix, pancreas	Betatene, Western Biotechnology Aqua Carotene LTD Cyanotech Corp., Nature Beta Technologies	[11, 12]
3	***Haematococcus***	*Haematococcus* contains high amount of astaxanthin (1.5-3% dry weight)	Astaxanthin is very effective in the treatment of Alzheimer's disease and Parkinson's disease	Cynotech Corporation, Parry Nutraceuticals, Fuji Health Science, Alga Technologies, and Aqua search Inc.	[13, 14]
4	***Aphanizomenon***	*Aphanizomenon* contains chlorophyll (1-2% dry	Can help to treat immunosuppression arthritis cardiovascular	Cell Tech International Inc.,	[15, 16]

No	Microalgae species	Salient features	Nutraceutical applications	Companies	References
		weight). It also produces polyunsaturated fatty acids (i.e., omega 3 and omega 6)	diseases mental health issue, and dermatological problems	AquaSource and Klamath Valley Botanicals, Inc.	
5	***Spirulina***	*Spirulina* is a rich source of nutrients such as B vitamins, phycocyanin, chlorophyll, vitamin E, omega 6 fatty acids *Spirulina* is 60-70% protein by weight	*Spirulina* has assisted in health areas like diabetes, and hypertension. *Spirulina* positively affects cholesterol metabolism by increasing HDL levels, which can lead to healthy cardiovascular functions	Puritan's Pride, Springtime Inc., Valley Naturals, Bio-Alternatives, and Watershed Wellness Centre	[17, 18]

3. Microalgal composition for health and wellness

In about 30,000 archived species of microalgae only few are harnessed for the production and utilization of biofuels and nutraceutical compounds (food and pharmaceutical applications). Nutraceutical compounds harness or concentrate the composition which is having high nutritive and medicinal value to provide healthy results. Some of the species having the composition (% dry weight of proteins, lipids and carbohydrates) together with their annual production and the leading countries in terms of production are shown in Table 2. There are various companies which are producing microalgal nutritional and pharmaceutical compounds in a viable manner and many products are being sold in the markets, some of them are shown in Figure 1.

Table 2: Microalgae composition for food and fuel with their production value[19, 20]

Microalgae	Protein (% dry weight)	Lipid (%dry weight)	Carbohydrate (% dry weight)	Annual production (tons dry weight)	Producer country
Chlorella vulgaris	51-58	14-22	12-17	2000	Taiwan, Japan
Dunaliella salina	57	6	32	1200	Australia, Israel
Haematococcus pluvialis	48	15	27	300	USA, India
Aphanizomenon flos-aquae	62	3	23	500	USA
Spirulina plantesis	46-63	4-9	8-14	3000	China, India

Figure 1: Commercialized products from different genera of microalgae. (a) Multivitamin from *spirulina* (b) Nutritional growth supplement from *chlorella* (c) Children growth granules from *spirulina* [21]

4. Conclusion

Microalgae is a potential feedstock which is an emerging source of food supplement and nutraceutical compound due to its rich composition (carbohydrates, proteins, and lipids) and its adjustable cultivation (in terms of pH, salinity and temperature). The paper provides a brief virtue of the efficacy of varied species of microalgae to produce food supplements and pharmaceutical compounds. The countries and companies producing microalgae at a large scale and then harvesting the biomass to obtain value added products are also incorporated with some of the commercialized products available in market. The capability of microalgae to treat/prevent serious ailments and provide nutritional food supplement for the ever-increasing population will provide the researchers a long road ahead to make the usage of microalgae economically, technically, and socially viable. The paper will hopefully contribute towards understanding the various health benefits of microalgae.

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Comparison of plant growth performance of water spinach, yard long bean and okra under different cropping system

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Abstract
Monoculture and polyculture cropping system have been widely practiced by farmers across the globe. Production, resource utilization and economic consequences are the main aspect of which farmers to consider. Monoculture has been applied by large commercial farmers as it offered highly productive output and efficient crop management. While polyculture often practiced by small farm as it offers structural and functional stability for agricultural production which resemble diverse natural tropical ecosystem term called agroecosystem. Field experiment was conducted to compare two type of cropping system by assessing the plant growth performance and yield of water spinach (*Ipomoea aquatica*), yard long bean (*Vigna unguiculata*) and okra (*Abelmoschus esculentus*). Each plant species sowed and planted on 15 rows of 3x6 ft planting box under polyculture and monoculture system. All plants were maintained by regular crop care and using organic farming method. The vegetable fruits and plants were harvested and collected once matured and the plant growth performance and yield data were collected and analyzed. Analysis of plant growth performance and total yield of water spinach show it performed better in monoculture compared to polyculture. The results suggested the selection of crop types polyculture together will have a major impact in productivity and plant growth. Further study on the selection of companion plants is crucial to identify crops that work symbiotically when cultivate together in polyculture.

Keywords: Cropping system, polyculture, monoculture, plant growth performance

1. Introduction

Global food demand by the growing population is increasing by the years putting pressure on global agriculture production [1, 2]. The growing demand urge the expansion agricultural land has causing simultaneous demand in reducing of it environmental impact on the ecosystem [1].

Current trend of agricultural intensification for high yields productivity by agricultural practicer has gave rise to negative impact toward natural biodiversity and it ecological function and services [3, 4]. Landscape simplification and specialized of agroecosystem by agricultural intensification aggravated biodiversity losses leading to reductions in ecosystem services on which agriculture depends [3]. Sustainable intensification of agriculture focused on existing land by utilizing technology improvements, adaptation and management practices may be one way to achieve both production and environmental

sustainability goal [5]. The idea uses natural ecosystem understanding to simulate and restore overall ecosystem functions and maintain or increase agricultural productions while minimizing the impact to the environment [6].

Natural ecosystem is reflected by high functioning community where available resources is utilized by diverse community presents in the system [7]. The types and level of natural ecosystem biodiversity in agricultural system may differ but the underlying ecological principle remain the same allowing modification of agricultural practice through crop diversity strategies to enhance agroecosystem [8].

The term monoculture by having only a one crop only at a time growing on a farm plot is a worldwide trend that practiced by many commercial farmer as it offers highly productive output, efficient and specialized management [9]. Although it have been recognize for it economic benefit, these systems have been criticized by ecologist because of their genetic and horticultural uniformity resulting loss of soil fertility and productivity, high pest pressure and rely on the use of massive amounts of agrochemical [10, 11]. The drawback of this system is the high risk of widespread crop failure due to high susceptibility to pest and disease resulting from crop uniformity [10].

In the tropics, polycultures have been an important component of small farm. The term polyculture describes the planting system of more than one crop in the same area in a particular of time [9]. This include mixed cropping, double cropping, crop association and intercropping. As it involved managing two or more species, it is not common practice in mechanized system due to greater complexity therefore it is and mostly practice in smallholder systems [4].

The rationale of ecological diversity of polyculture system is that it simulates the structural and functional stability of diverse natural ecosystem [9]. Extensive research has shown that increase diversity measured by greater plant community is positively related to higher levels of productivity [12] and improve agricultural sustainability [7]. Greater plant community allowed better utilization of land area and soil nutrient as it does not discriminate among different soil types [9].

Polycultures can result in interspecific competition and complementation depending on cultivar selection. This inhibitory or stimulating effects depends on the plant characteristic and it biological function which can be classified as amensalistic, comensalistic, monopolistic and inhibitory [20]. The critical decision of selecting cultivar in polyculture is to minimize negative competition and maximize positive complementation among species in the system. A mixed intercrop of corn-beans-squash (*Zea mays* L., *Phaseolus vulgaris* L., and *Cucurbita pepo* L., respectively) is a good example of complementary strategy. The different functional trait in between crop promote complementation by resource partitioning among the nitrogen-fixing beans, the low-growing squash that covers the soil and suppresses weeds, and the tall corn that acts as a trellis for the beans [13].

This biotic interaction could provide the self-regulated functions to the system designed and managed to enhance soil fertility without external inputs and crops protection against pests with compromising crop productivity [14]. Long term field study from 2009 to 2017 in the Pearl River Delta of China by [17] of soybean/sugarcane intercropping with reduced

nitrogen input in China found that the energy yields of sugarcane/soybean intercropping systems produce up to 40% higher compare to sugarcane planted in monocropping system. While carbon footprint value in unit yield (CFY) for sugarcane/soybean intercropping were down to 30% lower than those of the monocropping systems.

In this study, we compare the plant growth performance of water spinach, yard long bean and okra cultivate in two cropping system condition; monoculture and polyculture. We hypothesized that the cultivar mixtures would have a greater impact on plant growth performance as compared to monoculture cultivar.

2. Material and method

2.1 Study site

Open field experiment was carried out at biotic research farm of Universiti Teknologi Malaysia, Pagoh, Johor 2°09'18.8"N 102°43'57.8"E for a period of three months for one crop cycle of water spinach (*Ipomoea aquaticaokra*), (*Abelmoschus esculentus*), and yardlong bean (*Vigna unguiculata* subsp. *Sesquipedalis*) cultivated in two different cropping system - monoculture and polyculture. The study area is in tropical region with elevation of 134m above sea level. The average temperature is 27.5 °C with an average rainfall of 2125 mm/year. The site is palm oil plantation reclamation land with soil characteristic sandy loamy soil.

2.2 Experimental design

Seeds of the three crop were sown in seed trays using peat moss as planting medium. After five days of germination period, the seedlings were transplanted to 15 rows of planting beds (3 × 6 ft). The seedlings are planted either in polyculture or monoculture system. Fertilizer were applied once for every two weeks after planting using organic fertilizer NPK 3:3:3 with application rates of $9g/m^2$. Experiment was conducted in completely randomized block design with three replications per treatments**.** The fruits and plants were harvested once matured. The collected sample were measured for plant weight (g), root weight (g), root length (cm), plant height (cm), no of leaves (n), yield per harvest (g) and average fruit length (cm). One-way variance (ANOVA) and Tukey's HSD (THSD) multiple comparison test by SPSS were performed to determine significant difference between the treatments in term of plant growth performance parameter with mean compared at $p<0.05$.

3. Result and discussion

3.1 Plant growth performance: Monoculture vs polyculture

Table 1 shows the plant growth mean of water spinach cultured in monoculture and polyculture cropping system. Plant weight, root weight and root length showed significant different and this indicating water spinach grow better in monoculture system than in to polyculture. Functional trait of plant play important roles in determining it suitability for polyculture system. Uncomplemantary trait among crops in polyculture resulted in non-facilitation respond between cultivar species that cause asymmetric competition when one or more species suppresses the growth of another species. One example is size bias effect, a condition in which larger plants is more competative for resource competitive than smaller species [15]. Symbiotic nitrogen fixation is a good example of complementary fasilitation which both cultivars benefit on each other present. To achieve symbiotic

relation in polyculture it is important to understand each crop species functional trait, N-fixing ability and it photosynthetic pathway [16]. The ideal design of polyculture system is to reduce negative effect and increase complementarity among the cultivars but the setup may vary across temporal and spatial variablity [17]. Besides that, it is important also to identify methods or planting designs such as planting density that allow or even promote species coexistence [16]. The results suggested the intercropping with okra and yardlong bean suppressed the plant growth of water spinach.

Table 1: Water spinach: plant growth performance under different type of cropping system

Cropping system	Plant weight (g)	Root weight (g)	Root length (cm)	Plant height (cm)	Leaves number, n
Monoculture	97.22[a]	15.23[a]	22.36[a]	49.07[a]	60.04[a]
Polyculture	61.97[b]	8.78[b]	19.86[b]	47.36[a]	53.69[a]

Means in every column with similar letters were not significantly different at 5% level by THSD

3.2 Yield productivity: monoculture vs polyculture

Significant different were seen in the mean average fruit length (table 2) with yard long bean performed better in monoculture compared to polyculture. While analysis result of mean yield per harvest shows no different in between. The low intraspecific diversity in monoculture fields contributes to yard long bean perform better in term of fruit quality produces. Greater spatial and resource avaibility in monoculture tend to reduce competition in between species cultivar resulting better yield quality [18].

Table 2: Yard long bean: Yield performance and average fruit length under different type of cropping system

Cropping system	Yield (g) per harvest	Average fruit length (cm)
Monoculture	15.01[a]	37.22[b]
Polyculture	13.99[a]	34.34[a]

Means in every column with similar letters were not significantly different at 5% level by THSD

While okra shows no significant different for both mean yield and average fruit length. Weather variability have been observed to effect yield productivity in many different region [18]. Cultivars resistant and tolerance toward annual variation of rainfall and temperature may effect this study. Site condition such soil fertility also may have inhibit the ability of this study to detect the effect of different cropping system toward plant growth and yield performance. The availablity of N in soil have been shown to effect the study outcome of plant interactions study especially in short study period although some species have high tolerance in soil with low N concentration [16]. Site condition alone may not substantially effect the variability outcome in this as fertilizer and water management regime also contribute to the effect [18]

Table 3: Okra: Yield performance and average fruit length under different type of cropping system

Cropping system	Yield (g) per harvest	Average fruit length (cm)
Monoculture	16.43[a]	13.92[a]
Polyculture	18.00[a]	13.38[a]

Means in every column with similar letters were not significantly different at 5% level by THSD

Yield (g) per meter square for all crop as shown in table 4 indicates water spinach production is significantly higher in monoculture while okra have better yield in polyculture and insignificant mean different was observed in yard long bean yield. The 62% yield increase of water spinach in monoculture compared to polyculture may due to distinct functional traits of water spinach that perform better in less diversify agroecosystem. Functional traits specifically designed characterize an organism's response to the environment which relates to resource use by individuals [12]. An example of roots system dynamic for nutrient uptake significatly effect whole plant growth and ultimately its productivity. Study by [19] shows an improved maize yield by 30%-46% when intercrop with pea with numerous other study have also come up with similar pattern result. Understand crop functional traits in practising polyculture is the key factor in simulating natural functional ecosystem and producing better and higher yield. On ecological perspective, diverse functional community has greater resource partitioning, potentially utilizing the available resources more efficiently and ultimately increasing overall productivity and function as seen in okra total yield [12, 17].

Table 4: Yield (g) per meter square of water spinach (*Ipomoea aquaticaokra*), okra (*Abelmoschus esculentus*), and yardlong bean (*Vigna unguiculata* subsp. *Sesquipedalis*) under different type of cropping system

	Water spinach		Okra		Yard long bean	
	Mono	**Poly**	**Mono**	**Poly**	**Mono**	**Poly**
Yield (g) per meter square	1093.8[a]	697.1[b]	184.8[a]	202.5[b]	168.8[a]	157.4[a]

Means in every column with similar letters were not significantly different at 5% level by THSD
Note: mono=monoculture, poly=polyculture

4. Conclusion

Based on the data analysis, it can be concluded that water spinach is more suitable planted in monoculture. Okra performed better in polyculture and yard long bean can be planted in both cropping systems. The better yield of okra under polyculture suggested the systems enhances the plant growth of okra. The result indicated the selection of plant variety to be intercrop plays a crucial part in polyculture system. Responses of crop mixtures in a certain environmental conditions and stress may be responsible for the variation in plant growth each crop types. Right selection of cultivar that promote facilitation in polyculture might provide greater yield outcome especially under stressful environment [12].

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Assessment of monoculture and polyculture of water spinach (*Ipomoea aquatica)*, okra (*Abelmoschus esculentus)* and yard long bean (*Vigna unguiculata)* on soil properties

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Abstract
Nowadays, the practice of polyculture is a very common among farmers mainly for higher yield production and pest management. Thus, this study was conducted to determine the effect of monoculture and polyculture on the soil properties. There were four treatments involved with three replications for each of the treatment; T1: Monoculture water spinach (*Ipomoea aquatica)*, T2: Monoculture yard long bean (*Vigna unguiculata)*, T3: Monoculture okra (*Abelmoschus esculentus)* and T4: Polyculture of water spinach, yard long bean and okra. Analysis of moisture content, organic matter, pH and EC value for soil samples were carried out. The results showed that the polyculture has greatly improved the moisture content and the organic matter content in the soil. Analysis showed that the organic matter and moisture content for soil treated with T4 had the highest increment compared with other treatments. The results showed decrease in value for both EC and pH value for all treatment. The greatest decrease in pH value was in T2 whereas T4 showed the greatest decrease in EC value after the analysis. In conclusion, application of polyculture in agriculture practices does help in the improvement of the soil condition as compared to monoculture mainly in the aspect of organic content and soil moisture.

Keywords: Monoculture, polyculture, water spinach, yard long bean, okra

1. Introduction

Polyculture system which is also commonly known as intercropping is usually the practice of combining several types of plants on the same soil for producing maximum outputs in agricultural product. Besides, cultivating several plants in a region provides farmers a type of coverage where if one plant fails, there is another plants can be harvested. In contrast to monocultures, polyculture benefitted due to reduced fertiliser needs, high tolerance to herbivorous pests, and better soil quality due to the application of planting more than two types of crops [1]. There are many benefits cultivating vegetables with the multiple crops together such as higher commercial value, better agricultural practices and lower risk of failure. In multiple cultivation, good cultivation systems are needed, particularly on that selection of plants and mixed cropping designs [2].

Ipomoea aquatica (water spinach) is semi-aquatic perennial herbaceous plant that is easy to grow and is a very good source of protein, vitamins C and A and minerals, such as K,

Mn, Mg, and Fe. Water spinach usually has a quick growth period and is resistant to some of the insect pests and diseases. This is one of the reason water spinach is cultivated in many areas of country [3]. Okra (*Abelmoschus esculentus*) is commonly grown world wide due to its economical value and has been used as the intercopping crop along maize and bean. Okra has been often found to be planted in rows, with a gap between two consecutive rows of okra were recorded to be as big as 2 m [4]. *Vigna unguiculata* (yard long bean) is one of the most popular leguminous crops and widely cultivated throughout the South and Southeast Asian countries such as Malaysia, the Philippines, Indonesia. Yard long bean rich in protein and other nutrients such as potassium, iron, phosphorus, riboflavin and thiamine. Planting yard long bean helps to improve soil condition due to its nitrogen fixing properties with rhizobium bacteria [5].

2. Materials and methods

The location of the study was at ICA, UTM Pagoh. Monoculture and polyculture systems was applied in this field experiment by using three types of plants; water spinach, yard long bean and okra. The experiment was carried out using randomized block design with triplicate. There were four treatments: monoculture water spinach (T1), monoculture yard long bean (T2), monoculture okra (T3) and polyculture water spinach, yard long bean and okra (T4). The fertilization was applied using organic fertilizer (NPK 3:3:3) twice a week.

The data collection for soil moisture, organic matter, pH and EC were collected and analysed. Analysis was done using Statistical Packages for Social Sciences (SPSS). One-way of variance (ANOVA) and Duncan's multiple comparisons were performed to determine the differences among the fertilizer treatments in terms of the soil properties. Means comparisons between treatments were performed at $p \leq 0.05$.

3. Results and discussion

3.1 Effect of different types of plant cultivation systems on the soil moisture and organic matter

It was observed that the application of T3 resulted in soil moisture content 5.3% as compared to the T1 (5.2%). T2 has the lowest moisture as compared with all other treatments. The application of the polyculture (T4) had the highest increment which showed an increased by 37% compared to other treatments. On the other hand, the monoculture of yard long bean and monoculture okra showed an increased by 35% and 28%, respectively. Meanwhile monoculture of water spinach has the lowest increment which is by 8% only. This was given the fact that polyculture had a greater groundcover and shading effects than monoculture, decreasing the loss of water, thereby improving the conservation of moisture [6]. Soil treated with T1, T2, T3 and T4 showed an increase of 3.6%, 0.2%, 1.9% and 4.7% of soil organic matter, respectively. It is observed the soil organic matter was higher in soils of polyculture than monoculture. In comparison with other three types of the monoculture, water spinach (T1) showed the highest increment organic matter compared to other monoculture (T2 and T3). Higher root biomass production in intercrops of different plants might be the reason for increase of SOM level. The increased of root litter input in the soil can result in faster regeneration of the SOM pool with a relatively higher decomposition resulted in more soil organic matter [7].

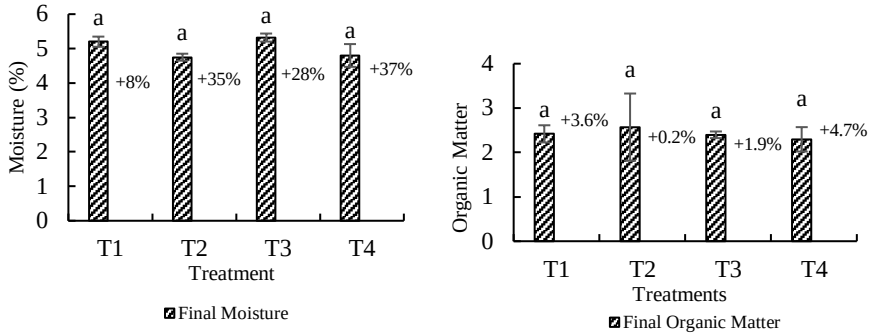

Figure 1: Effect of treatments on soil moisture (left) and soil organic matter (right). T1: Monoculture water spinach, T2: Monoculture yard long bean, T3: Monoculture okra and T4: polyculture water spinach, yard long bean and okra (T4). Means for treatment followed by the same letter are not significantly different (Duncan, P≤0.05)

3.3 Effect of different types of plant cultivation systems on the soil pH and EC

The soil pH and EC change was shown in Figure 2. The analysis revealed that different planting method led to a decreased of pH between 7.4% to 19.3% in all the treatments. The lowest soil pH reduction was in T1 and the highest soil pH reduction was in T4. The polyculture had the lowest pH value than the monoculture however there is no significant difference statistically. The pH of the soil is influenced by carbonates which the soil depends on the acidification of the soil. It can also influence by the environment or farming practices; acidification of the soil can lead to high losses of C from soil carbonates [8]. The uptake of cations and anions induced by the nitrification process contributes to the introduction of the proton to the soil, gradually reducing the pH content of the soil [9]. Polyculture (T4) showed decreased in EC value with the highest value 90% followed by T2, T1 and T3 with the percentage of decrease by 84%, 82% and 73%, respectively. The lower EC suggest a shortage of salt and high utilization of nutrient by plants [10].

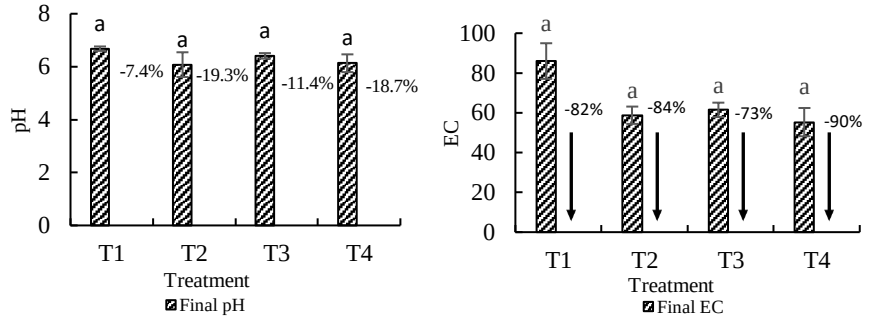

Figure 2: Effect of treatments on soil pH (left) and soil EC (right). T1: Monoculture water spinach, T2: Monoculture yard long bean, T3: Monoculture okra and T4: polyculture water spinach, yard long bean and okra (T4). Means for treatment followed by the same letter are not significantly different (Duncan, P≤0.05)

5. Conclusions

Polyculture significantly enhanced the soil organic matters and soil moisture contents as compared to the monoculture. In conclusion, practice of polyculture in agriculture land does help in improving the soil condition. Further research on the soil organic C level in long term polyculture farmland should be conducted to assess the long term impact of polyculture system fertilized with organic fertilizer.

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Potential of aquaponic system for the growth and production of strawberry (*Fragaria* sp.)

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Abstract
Aquaponics is the combination of aquaculture (fish) and hydroponic cultivation of plants. The good combination of both fish and plants will develop a sustainable aquaponic system in order to support the sustainable agriculture. A portable aquaponic system was designed to observe the potential of both *Clarias gariepinus* and *Oreochromis niloticus* from different tank in the aquaponic system against strawberry plant growth and production for ten weeks. Fish was fed with commercial diets ad libitum twice per day and the growth was recorded weekly. Strawberry leaves, flowers and fruit growth was recorded. Strawberry plants in the system of *C. gariepinus* starts flowering during week three and four with one flower respectively and it produced nine flowers during week nine and ten. While the plants only start flowering during week nine and ten with seven flowers respectively for treatment of *O. niloticus* tank. Plants in both treatments start fruiting during week nine with three and two fruits for *C. gariepinus* and *O. niloticus* tank system respectively. Both *C. gariepinus* and *O. niloticus* showed a significantly ($p<0.05$) weight gain percentage 116.84±2.35% and 88.57±1.91% respectively. The length increment of *C. gariepinus* (18.06±1.40cm) is more than *O. niloticus* (5.99±1.30cm). Fish survival are 100% for both *C. gariepinus* and *O. niloticus*. The combination of fish in both tanks and strawberry plants studied show growth increment. In conclusion, the aquaponic system designed for this *C. gariepinus*, *O. niloticus* and strawberry plants growing is efficient and possible to be applied either for urban farming or commercial farm production

Keywords: Aquaponic, *Clarias gariepinus*, *Oreochromis niloticus*, *Fragaria* sp., growth

1. Introduction

Aquaponics is an integrated system of tank-based, aquatic animal (typically fish) and hydroponic plant culture. Most of the nutrients needed for plant growth are derived from fish waste [1]. Because of its sustainability, aquaponics has gained increasing interest over the past several years. Nitrogen (in the form of ammonia and nitrate) and phosphorus (mainly in the form of phosphate) are the essential nutrients for plant growth and are found in aquaculture waste. By utilising them as a nutrient source, the plants filter dissolved waste products from the system, thereby reducing the need for biological or chemical filtration for water changes and water quality management to fish [2]. This soil-less agriculture system has been used to reduce pests and soil-borne diseases affecting monoculture crops. This aquaponics system stimulus the better use of land and water,

simpler methods of pollution control, improved management of productive factors, higher quality of products and greater food safety [3].

There are limitations associated with the prohibition of chemical fumigants for the control of phytopathogens in the cultivation of strawberries in the soil and the ergonomic difficulties of growing plants on the ground surface, both of which have hampered the recruitment of manpower [4]. Because aquaponic is an energy-efficient, prevent waste from being released into the environment, supply plants with organic fertilizers, reuse wastewater through biofiltration, and through multiple cropping ensure higher food production per unit area. It needs to be treated as a working model of green technology towards sustainability of aquaponic system in strawberry production [5]. Hence, this study was conducted to compare the potential of both *Clarias gariepinus* and *Oreochromis niloticus* in the aquaponic system against strawberry plant growth and production since these three species is highly demand in the local market.

2. Materials and methods

2.1 Plant and fish preparation

A total number of 60 *Fragaria* sp. daughter plants in the age of three weeks old were collected from Greenhouse 4, Politeknik Sandakan, Sabah. This plant locally called lowland strawberry which can grow in the temperature range of 25-35 °C. The mother plants were bought from Taman Agro-Fertigasi in Kajang, Selangor. A total of 30 healthy *Clarias gariepinus* and 30 *Oreochromis niloticus* juvenile in the length of 9-10 inches and 4-5 inches respectively was bought from fish hatchery at Politeknik Sandakan, Sabah.

2.2 Experimental setup and aquaponics design

In a locally built aquaponics module, one aquaponics system was built up using PVC pipe, iron frame, two fish tanks (250 L) for two different species, four wheels, two water pumps for each fish tank and twenty of plant pots (Figure 1). Coconut fibre was used as the growing substrate in plant pots. Plant pots used are 5 inches diameter and 6 inches height in size. Ten plant pots are connected to one fish tank.

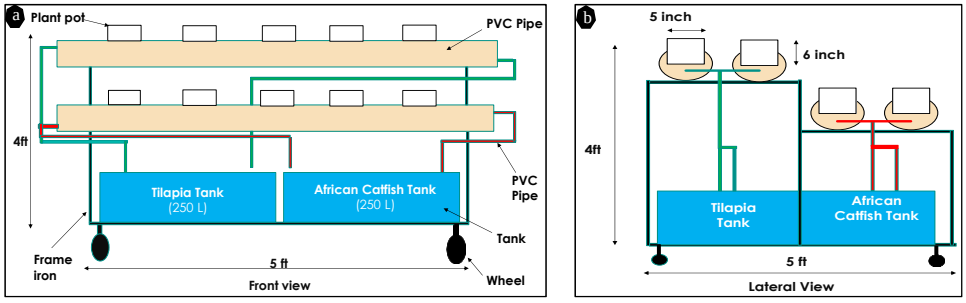

Figure 1: Aquaponic designation. (a) Front view of aqoaponic system; (b) Lateral view of aquaponic system.

The actual aquaponics system set up was shown in Figure 2. This system was set up in triplicates under a shaded open area at rain shelter pump house (Politeknik Sandakan

Sabah). All the fish in all treatments were regularly monitored at a frequency of three times daily for ten weeks. Fishes were fed with commercial diet (Star Feed brand) ad libitum twice per day. Uneaten food remained dissolve in the tank and providing a nutrient source for strawberry plants. Water from the fish tank was pump to the PVC pipe under plant pots as organic fertilizer sources for strawberry plants. Fish water quality was observed to maintain fish in good condition. Water in the fish tank was partially changed with dechlorinated tap water once per week.

Figure 2: Actual aquaponics system setup

2.3 Data collection and analysis

Growth of strawberry plants was observed in terms of the number of leaves grow, the number of flowers emerge, the number of fruits produce and survival rate (%). All the fishes from each tank were sampled once per week to measure their growth performance in term of weight gain (%), length increment (cm), survival rate (%) and specific growth rate (% day^{-1}). The water temperature, dissolved oxygen and pH was taken daily by using a digital thermometer, dissolve oxygen meter and pH meter respectively [6]. Data collected were subjected to T-test statistical analysis using SPSS software to identify the significant differences formed at 95% confidence level to show the differences means between the groups.

3. Results and discussion

Strawberry plants showed growth development for every week. Their plants keep growing but some leaves have become brown and dry. The death leaves were removed from plants. The new leaf growth increased week by week from an average of three to seven new leaf counted for strawberry related to both fish tanks (Figure 3). Strawberry plants grow in the tank of *Clarias gariepinus* had an earlier flowering which the on average the first flower emerged during week 3. However, this flower dry and drop off. There is more flower also grow in the tank of *Clarias gariepinus* during week nine and ten. Average of ten flowers recorded compared only seven flowers from strawberry plants grow in the tank of *Oreochromis niloticus*. Plants in both fish tanks starts fruiting in week nine where three fruits and two fruits emerged from plants related to *C. gariepinus* and *O. niloticus* tank respectively. All the strawberry plants showed a 100% survival rate (Table 1).

Table 1: Survival rate of strawberry plants

Aquaponic system	Survival rate (%)
African catfish tank	100.00±0.00[a]
Tilapia tank	100.00±0.00[a]

Values (Mean±SE) within the same column followed by same superscript were not significantly different at p<0.05

The growth of strawberry plants was photographed (Figure 4). Young leaf showed light green in colour. The strawberry flower is white in colour. Mostly plants grow in the tank of the *C. gariepinus* showed the development of runner or stolon with a new plantlet. However, this sucker was removed to avoid the reduction of strawberry fruit production. The fruits emerged is shown in Figure 4(d), where some of the fruits have irregular shape. As the fruit ripens, it was tasted and the fruits are sweet and slightly sour in taste as the plants are nature to lowland weather. Their taste was not influenced by the hot temperature like some of the commercial strawberry fruits that are sweet in the highland area with lower temperature but become very sour after reaching the lowland area. Strawberry fruits production and quality depends on temperature, photoperiod, and enough cooling time [7]. Not many fruits were collected and analyzed in this study because of the limited time. In comparison to the study by [4], their strawberry plants in the hydroponic system starts fruiting during week six after planting and the yield production was calculated until the week of 38th. But their study was using a chemical nutrient solution to fertilize their crop.

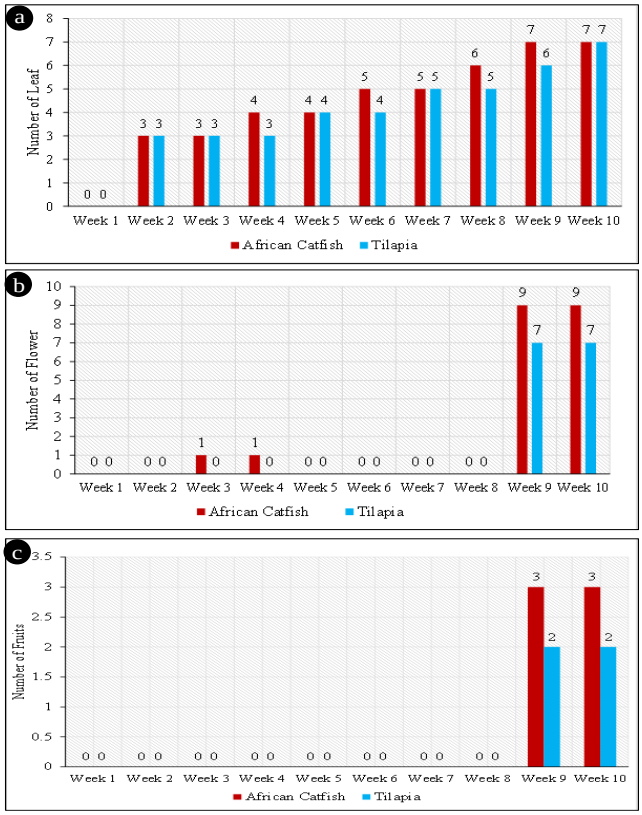

Figure 3: Strawberry plants growth performance in aquaponics system for different fish tank. (a) Average number of leaf grown; (b) Average number of flower emerged; (c) Average number of fruits formed.

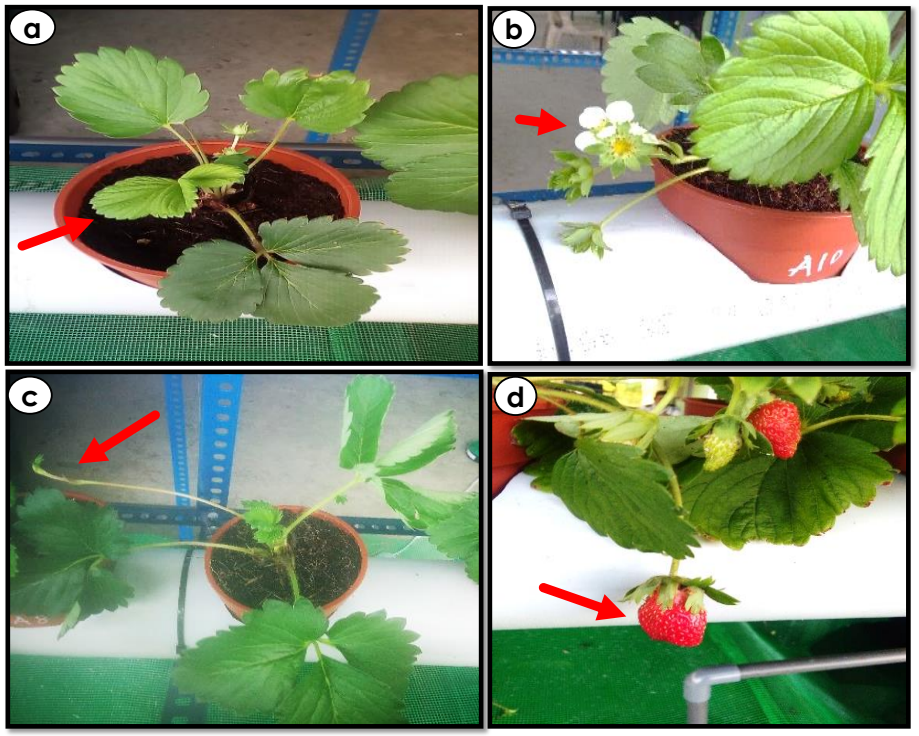

Figure 4: The strawberry plants. (a) New leaf growth; (b) Emergence of flowers (c) Emergence of runner; (d) Strawberry fruit.

Water quality parameters measured throughout this study were summarized in Table 2. Only three basic water parameters were measured in order to maintain the water quality. The dissolved oxygen was set in the range of 5.9–8.5 by recirculating water system using water pump. The water pH was in the range of 6.61–8.10. Temperature for the both tanks in this study was maintained in the range of 26.3–32.2 °C. These range of water parameter are favourable for both fish species to grow.

Table 2: Water parameters in fish tank

Water parameter	African catfish	Tilapia
Dissolved oxygen (ppm)	6.7 - 8.1	5.9 - 8.5
pH	6.61 - 6.95	6.86 - 8.10
Temperature (^{0}C)	27.3 - 32.0	26.3 - 32.2

Both *Clarias gariepinus* and *Oreochromis niloticus* showed positive growth in the consistency of weight and length increment (Figure 5). This aquaponics system is suitable for the growth of *C. gariepinus* and *O. niloticus* in the term of water quality. The favourable condition of tank culture influenced optimum fish growth rate.

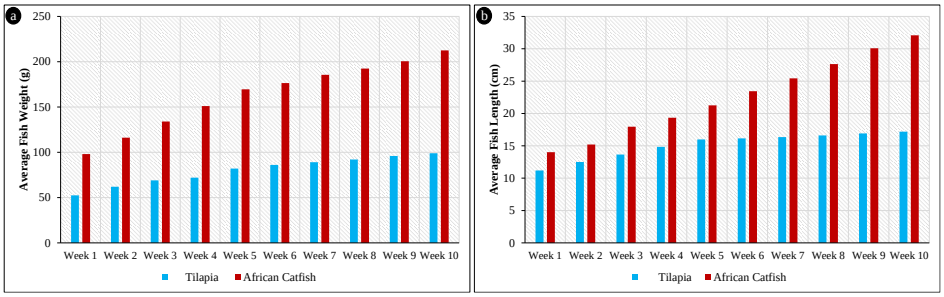

Figure 5: Growth of fish. (a) Average fish weight by week; (b) Average fish length by week.

These ten weeks of study revealed a significant growth increment of both *C. gariepinus* and *O. niloticus* (Table 3). The mean of initial and final fish weight and length was compared using T-test and results showed a significant different ($P<0.05$). The value of African catfish weight and length is higher than Tilapia as bigger size of fishes were used in the beginning of the study.

Table 3: Comparison of fish growth during the study (between initial and final)

Fish Growth	African Catfish		Tilapia	
	Initial	Final	Initial	Final
Average fish weight (g)	98.68 ± 1.99^{b}	212.50 ± 2.65^{a}	52.52 ± 1.87^{b}	99.04 ± 2.48^{a}
Average fish length (cm)	14.02 ± 1.12^{b}	32.08 ± 3.73^{a}	11.20 ± 1.90^{b}	17.19 ± 2.44^{a}

Values (mean±SE) with different superscript in rows under the same fish species are significantly different ($P<0.05$)

The growth performance of both *C. gariepinus* and *O. niloticus* was calculated using standard calculation formulae. The mean of weight gain (%), length increment (cm), survival rate (%) and specific growth rate (% day^{-1}) for both fish species were compared using T-test. All the results show no significantly different at $p<0.05$ (Table 4). This means both fish species possess the same average growth performance in this aquaponics system of strawberry. This aquaponics system is suitable to be used in both African catfish and Tilapia fish production.

Table 4: Comparison of fish growth in different tank

Fish growth	African catfish	Tilapia
Weight gain (%)	116.84 ± 2.35^{a}	88.57 ± 1.91^{a}
Length increment (cm)	18.06 ± 1.40^{a}	5.99 ± 1.30^{a}
Specific growth rate (% day^{-1})	1.67 ± 0.03^{a}	1.27 ± 0.23^{a}
Survival rate (%)	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}

Values (mean±SE) within the same row followed by same superscript were no significant different at $p<0.05$

4. Conclusions

The present study is more environmentally friendly without using chemicals as fertilizer to produce organic agriculture production. The combination of fish in both tanks and

strawberry plants studied showed positive growth. In conclusion, the aquaponic system designed for this *C. gariepinus*, *O. niloticus* and strawberry plants growing are efficient and possible to be applied either for urban farming or commercial farm production and contribute to sustainable agriculture. However, further investigation should be done on the water nutrient analysis in correlation with the time of the culture to make sure the strawberry plant growth can be more productive, and the crop yield can be evaluated.

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Immobilization of laccase enzyme on magnetically-separable hierarchically-ordered mesocellular mesoporous silica

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Abstract
Immobilized laccase was widely used as biocatalyst on different support material. In this study, laccase was immobilized in a magnetically-separable hierarchically-ordered mesocellular mesoporous silica (M-HMMS). Laccase was entrapped in M-HMMS before aggregating it with ammonium sulphate as the precipitant. Then, glutaraldehyde was used as a linker between the support and laccase. The parameters observed in this entrapped-crosslinked enzyme aggregate (E-CLEA)'s method was the concentration of crosslinker, temperature and pH to achieve high immobilization yield and high laccase loading. The results showed that the optimal immobilization conditions were at 0.5% (v/v) glutaraldehyde concentration of pH 5.0 and 25 °C which led to 93% of immobilization yield and laccase loading. The stability and magnetically behavior of M-HMMS enhanced the effectiveness of the laccase.

Keywords: Laccase, entrapped-crosslinked enzyme aggregates, mesoporous silica, immobilization, immobilization yield

1. Introduction

Laccase enzyme have been a promising tool to enhance advancements in paper and pulp, textiles, food, and wastewater treatment [1]. Extensive research showed laccases as sustainable and green biocatalyst which could degrade the phenolic compounds [2]. However, there were several disabilities of laccase which affect the production yield which poor operational stability, sensitive to changing conditions of the reaction environment especially in industry settings and high production costs of free laccase limit its applicability [3,4].

Hence, enzyme immobilization has been studied as one of methods that will help to enhance the operational stability of laccase [5]. Apart from entrapment of laccase, aggregation and crosslinking of laccase also being examined as the immobilization method. The combination process called entrapped-crosslinked enzyme aggregate (E-CLEA) by using magnetically-separable hierarchically-ordered mesocellular mesoporous silica (M-HMMS) as the support carrier apart from other support carriers such as alginate beads, chitosan beads, activated carbon, and nanoparticles [6,7]. Based on previous research, crosslinked enzyme in mesoporous materials lead to an active, stable and has high ability to load xylanase and cellulase than conventional physically adsorbed biocatalyst [8]. Therefore, in this study, we examined the best operating condition which are glutaraldehyde concentration, different temperature, and pH range by using E-CLEA's method and analysed the immobilization yield and protein loading as the final performance.

2. Materials and methods

2.1 Chemicals and enzyme

Laccase from *Trametes versicolor* (≥0.5 U mg^{-1}) and ammonium sulphate were purchased from Sigma Aldrich (St. Louis, MO, USA). 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was from Roche Diagnostics GmbH (Mannheim, Germany). Crosslinker used was glutaraldehyde solution (Grade II, 25% in H_2O) from Sigma Aldrich while Pluronic® P-123 ($(EO)_{20}(PO)_{70}(EO)_{20}$) was used to produce a hierarchically-ordered mesocellular mesoporous silica (HMMS). All other chemicals were of highest available purity.

2.2 Laccase immobilization by Entrapped-Crosslinked Enzyme Aggregate (E-CLEA)

The entrapped-crosslinked enzyme aggregate (E-CLEA) was conducted by laccase entrapment, aggregation with ammonium sulphate as precipitant and crosslinked using glutaraldehyde. 10 mg of M-HMMS was mixed with 1 ml of free laccase (0.5 U ml^{-1}) in a buffer solution (0.1 M acetate buffer at pH 4, 4.5 and 5) and incubated at 5 °C, 15 °C, 25 °C, 35 °C and 45 °C with agitation speed of 200 rpm for 1 hour. Then, laccase was aggregated with the addition of 2 ml cold ammonium sulphate as precipitant and incubated at 25 °C with agitation speed of 200 rpm for another 1 hour. After precipitation, the sample was crosslinked with 800 µL glutaraldehyde (0.1%, 0.3%, 0.5%, 0.7% and 0.9% v/v Glu/H_2O) in acetate buffer of pH 4.5. The solution then incubated for 6 hours at 200 rpm. Finally, the immobilized laccases were centrifuged (Hettich Zentrifugen Rotina 420R) and checked the supernatant's activity by using Genesys 10S UV-VIS spectrophotometer.

2.3 Laccase activity assay and immobilization yield

Laccase activity was measured using method published previously [9,10] with slight modification. The oxidation of 1 mM ABTS in pH 4.5 acetate buffer was monitored at 420 nm by using spectrophotometer. The reactions took 3 minutes after filled the cuvette with 1.5 ml buffer of pH 4.5, 1.35 ml ABTS solution and 0.15 ml of laccase solution. Time started as the laccase solution added into the cuvette. The activity was calculated by using Equation 1

$$\text{Laccase activity (U/min)} = \frac{\Delta A \; x \; V \; x \; 10^6}{\varepsilon \; x \; L \; x \; t} \qquad (1)$$

ΔA is the change of absorbance, V is the reaction volume, ε is the molar extinction coefficient (36000 M^{-1} cm^{-1}), L is the optical path and t is the reaction time. One unit of enzyme activity (U) is defined as the amount of enzyme required to oxidized 1 µmol of ABTS per min. While laccase immobilization yield was calculated by subtracting the activity of laccase recovered in the supernatant from the activity of initial laccase before immobilization. The equation for immobilization yield was constructed in Equation 2.

$$\text{Immobilization yield (\%)} = \left(\frac{Ui - Uf}{Ui}\right) x \; 100 \qquad (2)$$

Ui is the initial activity of laccase before immobilization while Uf is the final activity of laccase left in the supernatant.

2.4 Laccase loading

The amount of laccase loading in M-HMMS was calculated by subtracting the amount of laccase left in the supernatant from the amount of free laccase before immobilization [10]. The enzyme concentrations were determined by using Bradford with bovine serum albumin as the protein standard [5]. The equation for laccase loading was as Equation 3.

$$\text{Laccase loading (\%)} = \left(\frac{Pi - Pf}{Pi}\right) x\ 100 \qquad (3)$$

Pi is the laccase enzyme subjected to be immobilized and Pf is the laccase enzyme left in the supernatant after immobilization.

3. Results and Discussions

There were three parameters included in this study to achieve optimized immobilization condition which were glutaraldehyde concentration, temperature, and pH. The analysis of immobilization yield and laccase loading were observed.

3.1 Effect of different glutaraldehyde concentration on immobilization yield and laccase loading

Based on Figure 3.1, the highest percentage of immobilization yield and protein loading obtained were both 93% at 0.5% (v/v) of glutaraldehyde concentration followed by concentration of 0.7%, 0.9%, 0.1% and 0.3% with their respective immobilization yield and protein loading which were 92% and 91%, 89% and 88%, 82% and 82%, and finally 78% and 77%. The result shows that the highest percentage occur at 0.5% of glutaraldehyde concentration because of the optimum crosslinking between laccase to M-HMMS. While the lowest percentage because of the high reactivity and small size of glutaraldehyde penetrate the internal structure of laccase and react with amino residues that are crucial for laccase catalytic activity [5].

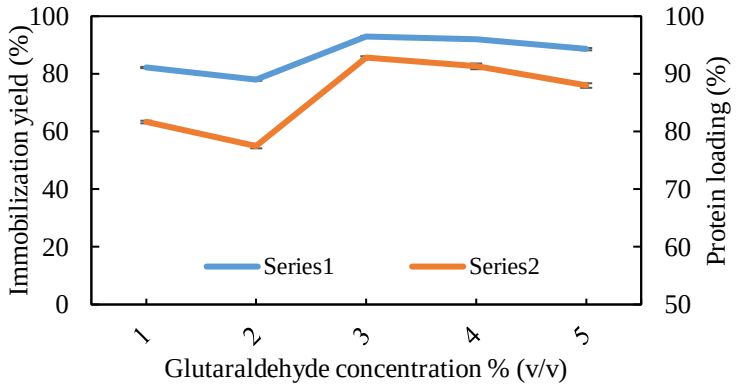

Figure 3.1: Percentage of immobilization yield and laccase loading in different glutaraldehyde concentration.

3.2 Effect of different temperature on immobilization yield and laccase loading

Figure 3.2 shows the percentage of immobilization yield and laccase loading from 5 °C up to 45 °C. The highest immobilization yield was achieved at temperature 45 °C with

98% and 97% of protein loading. While the lowest percentage yield and protein loading were obtained at temperature 15 °C with 84% and 83% respectively. Although the significant difference was ($P<0.05$) between temperature 45 °C and 25 °C, there are no additional cost needed to perform the immobilization process in the room temperature. Thus, it is cost effective and selected as the optimized temperature for laccase immobilization.

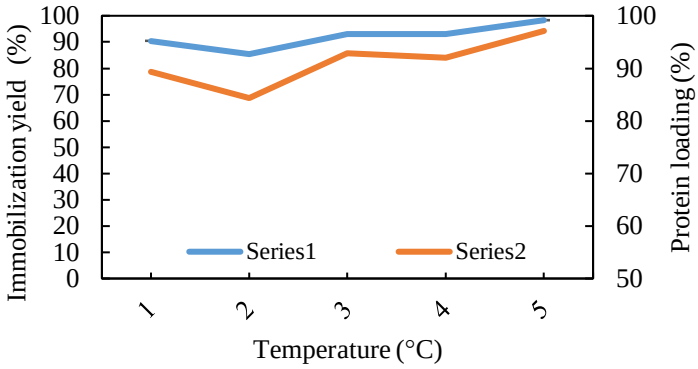

Figure 3.2: Percentage of immobilization yield and protein loading in different temperature.

3.3 Effect of different pH on immobilization yield and protein loading

The effect of different pH on immobilization yield and protein loading were compared and presented in Figure 3.3. It was found that the immobilization yield and protein loading were affected by the change in pH. The highest immobilization yield was recorded at pH 5 (97%) while the lowest immobilization yield was obtained at pH 4 with 91%. There is significant difference ($P<0.05$) between pH 5 and pH 4. However, between pH 4 and pH 4.5, there are no significant difference with ($P>0.05$). This indicates that the change in pH affected the immobilization yield.

The result for protein loading of pH 4 was the lowest compared to pH 4.5 and pH 5 with 90%, 95% and 96% respectively. Hence, the optimized condition for pH in laccase immobilization into M-HMMS was in pH 5. This is due to the maximum bond occur at pH with less acidic condition compared to pH 4 and pH 4.5 and due to electrostatic interactions with the matrix [11].

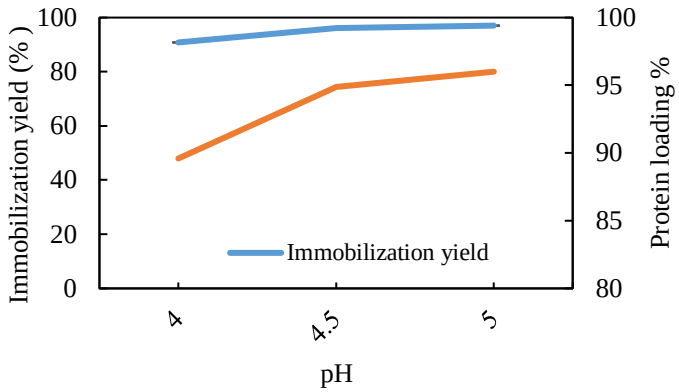

Figure 3.3: Percentage of immobilization yield and protein loading in different pH.

4. Conclusion

The result proposed that the immobilization yield and protein loading could be maximized using appropriate glutaraldehyde concentration and pH value. While there is no significant difference for different temperature thus 25 ℃ was chosen as the best operating condition for laccase immobilization along with 0.5% of glutaraldehyde concentration at pH 5. This process could be cost effective with maximum yield of production. Future works is warranted to examine more immobilization parameters and analysis to obtained higher immobilization yield and protein loading with precise results.

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Internet of Things for indoor environment monitoring of swiftlet farms

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Abstract
The swiftlet farming industry has reached billions of dollars in value, due to the high demand of edible swiftlet bird nest. Referred as "caviar of the east", edible bird nests (EBN) are produced by swiftlets from their saliva that are built at high and dark corners of natural limestone caves. Man-made swiftlet houses which closely resembles natural cave habitats have been constructed as breeding structures for swiftlets, to ease the process of harvesting the raw EBN. In order to produce high quality and pure EBN, ranchers monitor and control the swiftlet house environment. However, most of the environmental parameters monitoring and measurements are currently implemented manually using conventional systems in situ. Herein, we proposed an Internet of Things (IoT) system for swiftlet farming as a model for the swiftlet ranchers to ensure better monitoring of in-building environmental parameters for the swiftlets. To achieve this aim, we discuss indoor environmental factors for swiftlet house, such as air and surface temperatures, and relative humidity. Finally, a system has been developed to sense and monitor all the important parameters automatically in real-time and remotely, where the data can be monitored via the web. The system is capable of monitoring the associated environmental parameters to enable optimal environment for man-made swiftlet houses. The designed system is more effective than the traditional method in the terms of data collection time saving and real time monitoring.

Keywords: In-building environment monitoring, swiftlet farming environment, Internet of Things

1. Introduction

Swiftlet farming industry value has reached billions of dollars due to the high demand of edible swiftlet bird nest. The edible swiftlet bird nest (EBN) comes from the saliva of swiftlets namely the White-nest swiftlet (*Aerodramus fuciphagus*) and the Black-nest swiftlet (*Aerodramus maximus*), which are small natural limestone cave-dwelling insectivorous birds. The nutritional and medicinal properties of EBN, has been summarized by [1]. EBN are rich with nutritious minerals that rejuvenates the human body such as calcium, phosphorus, iron, sodium, potassium, iodine and eighteen types of amino acids.

As the number of caves EBN is dwindling due to over-harvesting and it is not enough to meet the current demand, therefore most of the supplies of EBN has been mainly sourced from man-made swiftlets houses. Herein, the swiftlet nesting house environment needs to be monitored and controlled to ensure optimal swiftlet cave-like habitat is achieved. The

Internet of Things (IoT) has shown that various agricultural farming environment can be achieved through automated monitoring and control of optimal environment to achieve higher yield via precision farming [2]. From our literature review, presently, there is a lack of literatures on IoT system deployed for swiftlet farming. This paper elaborates on the design of an IoT system for indoor swiftlet house monitoring and the monitoring performance of the IoT system in a real swiftlet house environment.

2. Materials and methods

The importance of right habitat and suitable environmental conditions for the swiftlets to produce EBN in swiftlet houses has been highlighted by [3]. Within the house itself, indoor environmental factors such as air and surface temperatures, relative humidity, air velocity and light intensity are critical for high quality EBN production. Herein, a natural cave-like environment should be provided in order for swiftlets to occupy and build their nest in the houses. Below is the summary of two main environmental factors, their optimal range and their impact towards EBN production [3-6].

Table 1: Temperature and humidity optimal ranges and its implications

In-building environmental parameters	**Optimal Range**	**Implications of environmental parameters towards swiftlet**
Air and surface temperatures	25 °C - 35 °C	High temperature will damage the eggs, whereas low temperature harms young featherless swiftlets.
Relative humidity (usually humidifiers and water pools are used to maintain humidity levels)	80% - 90%	High relative humidity leads to fungal growth in the nests, swiftlets reluctant to nest on fungus covered surfaces. Good humidity ensures swiftlet nest do not crack. Low humidity affects adhering ability of the nest to the wall surfaces and nest falls easily.

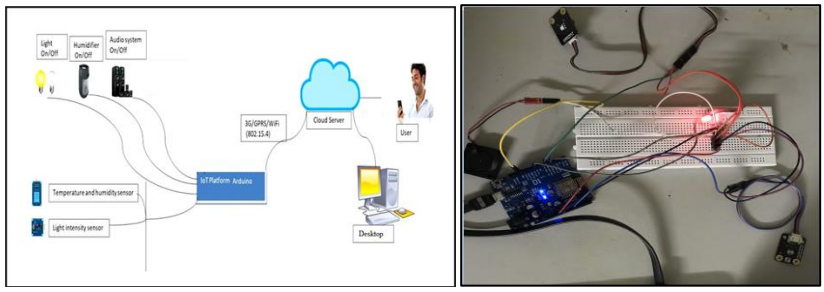
2.1 IoT system for swiftlet farm monitoring

Herein, we have developed an automated IoT system to monitor the indoor environment. It is able to replace the monitoring tasks done by farmers measuring humidity and temperature using handheld instruments in situ once in every three or four hours, every day. In addition, manual tasks to activate and deactivate the humidifier and audio sound systems (indoor and outdoor swiftlet sound to attract male and female swiftlets to nest) can be done remotely as scheduled and needed. The proposed system for monitoring (and controlling) the critical parameters in a swiftlet farm is shown in the Figure 1 (a) below.

2.1.1 IoT end user node

The IoT end user node is equipped with humidity, temperature and light intensity sensors that controlled by a microcontroller unit (MCU) as shown in Figure 1 (b). Herein, the AM2302 Digital Humidity Temperature DHT22 sensor with an analog to digital converter is connected to the WeMos-D1 MCU. The DHT22 sensor are made of two parts i.e. a capacitive humidity sensor and a thermistor. In addition, the WeMos-D1 MCU,

which is an Arduino based MCU with an ESP8266-12 chip that enables WiFi connectivity within the same MCU board.

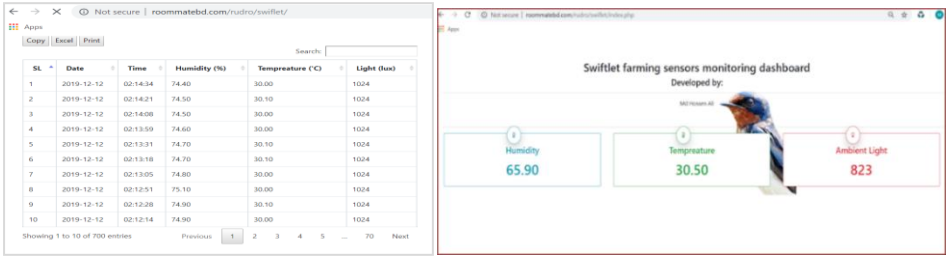


(a) (b)

Figure 1: IoT system for swiftlet farm monitoring (a) System components; (b) IoT nodes for measuring environmental parameters

2.1.2 Real-time data storage and visualization

The system is able to perform humidity and temperature measurements inside the swiftlet farm automatically where the data is stored in the cloud as in Figure 2 (a). The IoT node sends all the information via a MQTT protocol to the cloud using a WiFi connection that is linked to a 3G mobile network. The visualization of the data can be accessed real-time in a simple dashboard shown in Figure 2 (b).



(a) (b)

Figure 2: IoT cloud data storage (a) Snapshot of the cloud database; (b) Online real-time monitoring dashboard.

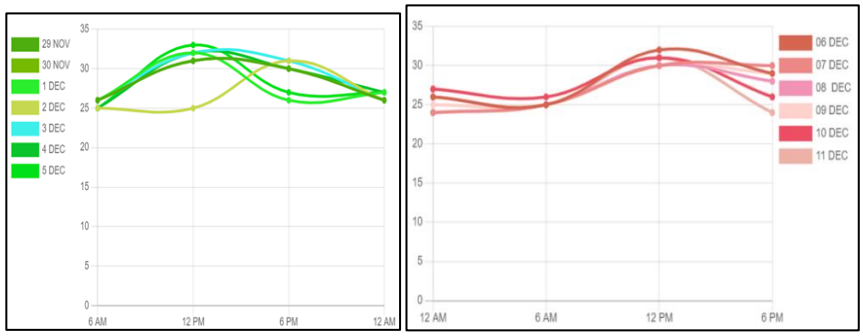
3. Results

In order to test the efficiency and effectiveness of the developed system, real time deployment have been conducted in a swiftlet farm located at Pusat Bandar Country Homes, Rawang, Selangor. Six days data monitoring of humidity, temperature and light intensity parameters has been performed and analysed to determine whether the system is giving similar findings in comparison to the manual in situ measurements.

3.1 Temperature readings

The swiftlet house minimum temperature and maximum temperature was 25 °C and 33 °C respectively as shown in Figure 3. The IoT system result shows that it is consistent with the in-situ measurements taken. As elaborated earlier the suitable temperature of swiftlet habitat is 25 °C-35 °C. If temperature readings are less than 24 °C, this can cause death

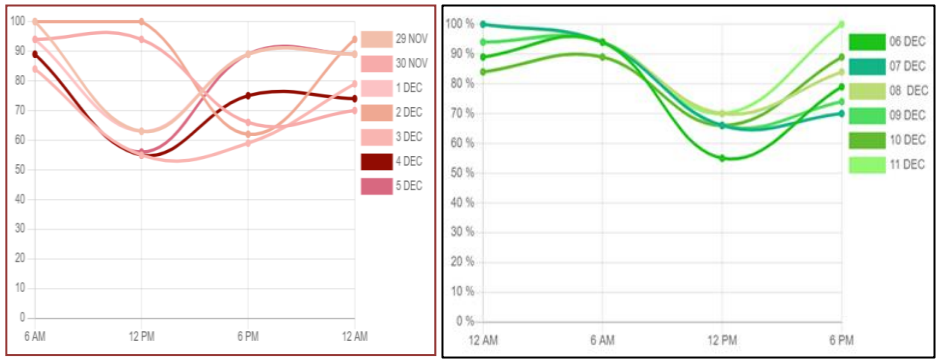
to young swiftlets and if the temperature rises more than 35 °C, the birds will not nest in the man-made structures.



(a) (b)

Figure 3: Temperature readings (a) Manual readings (29 Nov, 2019 to 05 Dec, 2019); (b) IoT System readings (29 Nov, 2019 to 05 Dec, 2019)

3.2 Humidity readings



(a) (b)

Figure 4: Humidity readings (a) Manual readings (29 Nov, 2019 to 05 Dec, 2019); (b) IoT System readings (29 Nov, 2019 to 05 Dec, 2019)

The suitable humidity for swiftlet birds is 80-90 % according to our established literatures. The range of humidity measurements have been shown in Figure 4, where the humidity drops significantly at noon time and improves as dawn arrives. The humidifiers are timed to operate at noon time to counter the warming effect in the swiftlet house that significantly lowers the required humidity of the system. We can also conclude that if a humidity control mechanism based on the IoT system is deployed, the optimal humidity range which is between 80-90% can be achieved; by turning on and off the humidifier correspondingly, when the humidity drops and is too high (nearly 100%).

4. Conclusions

The IoT monitoring system for the swiftlet farm have been tested and the system has shown to produce consistent results with the in-situ measurements. It has shown the capability of sending measurements of temperature and humidity, within a regular interval in real-time. Internet of Things system for the swiftlet house has enabled autonomous

measurements with a higher reliability, where the system operates automatically without the need to manually go to the site for measuring. The data can be viewed via the web in real time. Simulation of controlling the humidifier was also implemented but was not reported here.

Acknowledgments
The swiftlet monitoring IoT system was implemented by Mr Md. Hossen Ali from his MSc. research project, where the results of this paper was obtained from.

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Effect of different pre-treatment methods on physical properties and aflatoxin accumulation of dried chili (*Capsicum annuum*)

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Abstract
A vacuum tray dryer (VTD) was used to dry *cili kulai* or *Capsicum annuum*. Different types of pre-treatment methods such as treated with citric acid, blanching and combination of blanching with citric acid solution were used to study the physical properties and aflatoxin accumulation of dried chili under vacuum drying condition. Evaluation of physical properties such as moisture and accumulation of aflatoxin were compared with untreated chili before drying in VTD. All pre-treatment techniques not showing any significant differences ($p<0.05$) between different pre-treatment methods on the moisture contents of dried chili. The moisture contents of dried chili were below than 12% for all treatments after drying in VTD. All the pre- treatments showed significant value in moisture content compared to the control sample. The chili pre-treated with combination of blanch and citric acid had the lowest moisture content compared to the rest. All dried sample drying with VTD of untreated and treated samples are free from aflatoxin. In conclusion, chili pre-treated with the combination of blanching and citric acid solution before drying in VTD was the lowest in moisture content and given product with higher quality.

Keywords: Dried chili, vacuum tray dryer, pre-treatment, moisture, aflatoxin

1. Introduction

Chili (*Capsicum annum)* is one of the vegetables widely used in Asian countries commonly as spice product in cooking [1]. Dehydration removes moisture and stops the growth of bacteria, yeasts and molds that normally spoil the food. Vacuum drying method used high pressure and low temperature for drying which may help improve the qualities such as colour, shape, and nutritive values of the dried product. In addition, bioactive compounds in dried products also can be maximized by using the pre-treatment methods [2]. Therefore, the objective of this study was to investigate the best method of pre-treatment on the physical quality and aflatoxin accumulation of dried red chili under vacuum drying condition.

2. Materials and methods

2.1 Raw materials

Fresh red chilies (*Capsicum annum* L.) variety of Kulai were purchased from a local distributor in Pagoh, Johor, Malaysia.

2.2 Effects of pre-treatments on quality of dried chili
There are four types of pre-treatment involved; control (untreated sample), blanching, citric acid and blanching added with citric acid. The chilies were blanched in boiling water for 3 minutes and immediately cooled in ice water for three minutes. All the chilies were dried in vacuum tray dryer at temperature 50ºC under pressure at 0.28 mbar until reached constant weight [3].

2.3 Vacuum drying methods for drying of chili
Vacuum tray drying was conducted at a temperature 50 °C and at atmospheric pressure 0.28 mbar [5]. The samples were dried until they reached a constant weight. The dried samples were then packed and sealed in airtight packaging and keep in desiccator before the analysis.

2.4 Analysis

2.4.1 Moisture content
The moisture content was determined by weighing 5 g of chili sample in the drying dish and placed in oven for 3 hours at 105 °C until reached constant weight. Then, the dried weight of the sample was recorded and the moisture content was calculated [2].

2.4.2 Aflatoxin determination
Aflatoxin quantities of standards and samples were determined using HPLC with fluorescent detection. Aflatoxin contents were determined by comparing peak areas with those obtained from reference solutions by HPLC analysis [4-5]. Ground sample (25 g) and sodium chloride (5 g) was weighed and inserted into a one-liter capacity, solvent resistant blender jar. A volume of 100 ml of 80% methanol was added and blend at high speed for 2 minutes. The sample was filtered through Whatman paper no. 4 filter paper and centrifuged at 4,000 rpm for 10 minutes. 2 ml of filtrate was diluted with 20 ml of 10% Tween 20 in phosphate buffered saline (PBS). The pH was adjusted to pH 7.4 using 2M sodium chloride.

2.4.3 Data analysis
The data were analyzed by ANOVA and Duncan's multiple-range test at 5% probability level ($P < 0.05$) using SPSS statistics software (Version 21.0, SPSS IBM Corporation, USA).

3. Results and discussion

3.1 Effect of pre-treatment on moisture content and aflatoxin accumulation under vacuum drying condition
In this study, the quality of the dried chili under vacuum drying conditions was evaluated. The study conducted to study the influence of vacuum drying method on product quality which is measured primarily by means of moisture content and aflatoxin. Considering all quality criteria, drying using different types of pre-treatment had significant influence on the quality parameters of dried chili (Table 1).

3.1.1 Moisture content of dried chili
Drying under the same condition for fresh chilies treated with different pre-treatment techniques can decreased moisture contents of chilies after drying in VTD when

compared with untreated sample. As shown in Table 1, combination of pre-treatment; blanching and citric acid gave the lowest moisture content than the other pre-treated samples. The moisture content of dried chilies for all treatment varied from 7.48% to 8.90% which is below than the accepted range as recommended by Thai Industrial Standards Institute (TISI 456-1983) [2] when compared with untreated samples (control) about 10.89% when drying by VTD technique. Generally, there is no significant differences ($p<0.05$) between different pre-treatments to the moisture content of dried chili when drying under VTD method.

3.1.2 Aflatoxin accumulation of dried chili

The results for aflatoxin in all samples were found not detected including the untreated samples. Previous study had been carried out on few genotoxicity assays were executed on capsicum, and they found out that the toxicity was very low when chilies dried under good drying condition [1].

Table 1: Effect of different types of pre-treatment on moisture and aflatoxin contents when drying with VTD

Pre-treatment	Moisture (%)	Aflatoxin (%w/w)
Control	10.899 ± 0.38^{b}	nd
Blanch	7.515 ± 0.09^{a}	nd
Citric acid	8.905 ± 1.25^{a}	nd
Blanch + citric acid	7.475 ± 0.97^{a}	nd

nd denotes not detected. Statistical analysis showing different letter on the same column are significantly different at $p < 0.05$

4. Conclusion

In this study, *Capsicum annuum* pre-treated with the combination of blanching and citric acid solution before drying in VTD was the lowest in moisture content and given product with higher quality.

Acknowledgement

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Evaluation of the antioxidant and antimicrobial activities of leaves and fruits extracts from *Garcinia atroviridis*

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Abstract
Garcinia atroviridis (asam gelugor) is native in Malaysia and has been used in culinary and traditional medicine for years. This study was designed to evaluate antimicrobial and antioxidant activities of water extracts of the leaves and fruits of *G. atroviridis.* The antimicrobial activities were evaluated against *Staphylococcus aureus* and *Escherichia coli* using the agar-well diffusion method. The result showed that fruits and leaves extracts inhibited *S. aureus* and *E. coli* in dose-dependent manner. The fruits extract showed the highest inhibition activity against *S. aureus* (47.3 mm) and E. coli (44.7 mm) at a concentration of 1 g/mL. The antioxidant activity of extracts was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. All extracts tested were able to reduce DPPH radicals. The fruits extracts showed higher activity than leaves extract with IC_{50} of 46.57 μg/ml and 408.33 μg/ml, respectively. In conclusion, the fruits extract has a better antimicrobial and antioxidant activities as compared to leaves extract of *G. atroviridis*.

Keywords: *Garcinia atroviridis*, antioxidant, DPPH, antimicrobial

1. Introduction

The imbalance of oxidant and antioxidant equilibrium in the body can cause oxidative stress that leads to damage of the cells in an organism[1]. The cells of body and organs systems need to be protected against reactive oxygen species (ROS). ROS and other forms of free radicals formed during metabolism or through the action of ionizing radiation in a living organism and can attack healthy cells in the body. Thus, a healthy cell will lose its function and structure [2]. Free radicals can be defined as chemical species with an odd or unpaired electron. They are short-lived, unstable and highly reactive to pair up the odd electron and finally achieve a stable configuration. They are the major contributors to ageing degenerative diseases such as cancer, cardiovascular disease (CVD), diabetes and other illness [3]. Antioxidant, the substance that can protect the cells and organ system against ROS by counteracting the free radicals. Several studies have shown that parts of *Garcinia atroviridis* such as root, leaf, trunk and stems bark extract are relatively abundant sources of antioxidant components with good potential antioxidant properties [4-7].

G. atroviridis also known as 'asam gelugor' is widely used in traditional medicines in South East Asia. The dried fruit rind is used in cooking as seasoning or flavor additives [8]. The locals believe that the dried fruit rind can treat various diseases like ear-ache, cough, throat irritation, dandruff, improving blood circulation and as laxative [9],

meanwhile the leaves are used to steam the fresh fish to delay spoilage [10]. Phytochemical studies on *G. atroviridis* have reported the presence of garcinia acid (hydroxycitric acid) and its lactone, xanthone (atroviridin, atrovirisidone and atrovirinone) as well as organic acid, flavonoids, benzophenone [11-12]. Previously, *G. atroviridis* fruits and leaves extracts demonstrated various *in vitro* biological functions including antioxidant, antimicrobial and antibacterial and antitumor-promoting activities while being non-toxic [10, 12-13]. Basri *et al.* [14] reported that ethyl acetate extract of *G. atroviridis* generally exhibited strongly of antimicrobial activity against 7 bacteria strains and 2 yeast strains. However, antioxidant and antimicrobial activities of the water extracts is not clear. Therefore, the objective of this study is to determine the antioxidant activity of the water extracts of leaves and fruits of *G. atroviridis* using DPPH radical scavenging assay. This study also examines the antimicrobial activity of the extracts against human pathogens (*Escherichia coli* and *Staphylococcus aureus*) by agar well diffusion method.

2. Material and methods

2.1 Research materials

The fruits of *G. atroviridis* were obtained from a local orchard in Muar, Johor. Meanwhile the leaf powder of *G. atroviridis* was obtained from a company in Sungai Buloh, Selangor. 2, 2-diphenyl- 1-picrylhydrazyl (DPPH) and ascorbic acid were purchased from Sigma Aldrich (St Louis, MO, USA). The antimicrobial activity of *G. atroviridis* extract was assessed against bacteria species; Gram-positive (*S. aureus,* ATCC 2592*)* and Gram-negative (*E. coli,* ATCC 35218). All the other chemicals and solvents used for the analysis were of analytical grade

2.2 Extraction of G. atroviridis

All fruits were cleaned and checked to remove any damage, disease or pest infected fruits. The fruits were cut to small pieces and were dried in an oven (Memmert GmbH, Schwabach) at 45 °C for a few days until the moisture removed. Then, the dried leaves and fruits were ground into small particles powder. 30 g of *G. atroviridis* powder for fruits and leaves were placed in a 250 ml beaker and was added with 100 ml of the distilled water. The extraction was carried out by using sonicator with water bath (Daihan Scientific, Korea) at 60 Hz for 10 min at 25 °C and filtered. Afterwards, the treatment for the extraction of *G. atroviridis* was slightly modified and done according to Jayaprakasha et al [15]. Then, the filtrated was treated with 120 ml ethanol to remove the pectinaceous material and the mixture was centrifuged at 2000 rpm for 15 min. The supernatant was then concentrated under reduced pressure using a rotary evaporator and stored at 4 °C until further used.

2.3 DPPH radical scavenging activity

100 µl of the leaves and fruits of *G. atroviridis* extract with different concentration starting at 4 mg/ml and 1 mg/ml, respectively, were mixed with 100 µl of 0.1 mM 2, 2-diphenyl-1-picrylhydrazyl (DPPH) methanolic solution in 96-well microplate. Then, the mixture was incubated in room temperature for 30 minutes in the dark condition. The absorbance of the mixture was measured using an ELISA (Versamax Microplate Reader, US) at 517 nm. Ascorbic acid was used as a standard. Inhibition of free radical DPPH in per cent was calculated as follows:

$$\text{Inhibition (\%)} = 100 - 100\ (A_s / A_o)$$

Where (A_o) is absorbance of the blank and (As) is absorbance of the sample at 517 nm. The IC_{50} value was calculated and evaluated to obtain the concentration needed to inhibit 50% of the scavenging activity. The lower the IC_{50} value, the stronger the antioxidant activity.

2.3 Anti-microbial activity

The agar well diffusion method on Mueller-Hinton agar (MHA) was applied according to the method described by Sonia *et al.* [16]. The determination of antimicrobial activities of the *G. atroviridis* extract was against *S. aureus* and *E. coli*. The bacteria culture was diluted with sterile physiological saline solution with reference to the 0.5 McFarland standards to provide an optical density comparable to the density of a bacteria suspension 1.5 x 10^8. A 5 ml portion of this inoculum was placed onto the surface of nutrient agar plates and was allowed to remain in contact for one minute. The excess inoculum was removed using a sterile syringe and the plates were allowed to dry for 20 minutes at room temperature. Briefly, MHA agar plates were inoculated with bacterial strain under aseptic conditions and wells (diameter = 6 mm) were filled with 20 µl of the *G. atroviridis* extract. Then, the plates were incubated at 37 °C for 24 hours in an incubator (Memmert, Schwabach). The diameters of the inhibition zones were measured in millimeters. A zone of inhibition was compared with the standard antibiotic streptomycin (10 µg/disc), whilst a blank disc impregnated with the methanol was used as the negative control

2.4 Statistical analysis

All analysis was carried out in three replicate measurements and results were subjected to statistical analysis. Statistical analyses were performed using Analysis of Variance (ANOVA) test ($\alpha = 0.05$) and analyzed using Microsoft Office Excel 2010 and IBM® SPSS® Statistic Server version 20.0. Experimental results were expressed in mean ± standard deviation (SD).

3. Result and discussion

3.1 DPPH scavenging activity

Table 1 represents the scavenging activity of ascorbic acid, *G. atroviridis* fruit water extract (GAFWE) and *G. atroviridis* leaf water extract (GALWE) against free radicalDPPH). The effect of the *G. atroviridis* extract on DPPH radical scavenging activities was determined based on their hydrogen donating ability [16]. The lower IC_{50} value indicates a high antioxidant activity. The value of IC_{50} for ascorbic acid was recorded in Figure 1 was 2.0 ± 0.001 µg/ml showed a greater ability to inhibit DPPH radical. Meanwhile, compared to GAFWE presented lower antioxidant activity with an IC_{50} value of 46.57 ± 0.93 µg/ml. However, DPPH radical scavenging activity of GWLWE was significantly high with an IC_{50} value 408.33 ± 9.43 µg/ml. The antioxidant results are in agreement with Al-Mansoub *et al*. [5], which revealed that the GAFWE showed higher antioxidant value than GALWE. Previous studies documented that *G. atroviridis* fruit acids, including tartaric acid, ascorbic acid, citric acid, and hydroxycitric acid (HCA) [17] contribute to its antioxidant activity [18]. The flavonoids presence in the leaf has been effect of antioxidant activity [10, 19]. Besides, xanthone which include several potential antioxidants such as atroviridin, atrovirisidone, atrovirinone and garcinol have also been isolated from parts of *G. atroviridis* [20-23].

Table 1: The IC_{50} value for optimized *G.atroviridis* extracts

Extract/Standard	Absorbance 517 nm concentration at IC_{50} (µg/ml)
GAFWE	46.57 ± 0.93^{b}
GALWE	408.33 ± 9.43^{c}
Ascorbic acid	2.0 ± 0.001^{a}

Values are expressed as mean ± SD (n = 3)

3.2 Agar well diffusion method

The antimicrobial activity and yield of *G. atroviridis* extracts is presented in Table 1 and Table 2. After incubating at 37 °C for 24 h, the result showed that fruits and leaves extracts inhibited *S. aureus* and *E. coli* in dose-dependent manner. The fruits extract showed the significantly highest ($p<0.05$) inhibition activity against *S. aureus* (47.3 ± 2.3 mm) and *E. coli* (44.7 ± 0.6 mm) at a concentration of 1 g/ml. Meanwhile the leaves extract give inhibition activity against *S. aureus* (35.7 ± 1.5 mm) and *E. coli* (36.3 ± 1.2 mm) at same concentration. *E. coli* is the common bacteria in gastrointestinal tract in human [10]. Meanwhile *S.aureus* frequently found on the skin [10]. It is known that the life span of antibiotics is limited, hence new sources of antibiotic are required especially from plant source. *G.atroviridis* can be one of the medicinal plants used in various traditional and alternative remedy to treat human disease. It shows *that G.atroviridis* extract against human bacteria pathogenic strains demonstrated the ability to inhibit the growth of the microorganism. These findings were in agreement with other reports that revealed the antimicrobial activity in *G.atroviridis* in different solvents of extraction and different part of *G.atroviridis* against bacteria and yeast strain which exhibit antimicrobial activity [9], [14], [24].

Table 2: Antimicrobial properties of GAFWE using agar-well diffusion method. Zone Diameter Inhibition (mm)

Bacterial strain	Fruits extracts (g/ml)			Streptomycin	Negative control
	1	0.5	0.1	10 µg/disc	
S. aureus	47.3 ± 2.3^{a}	40.7 ± 0.6^{b}	26.3 ± 0.6^{c}	18.00 ± 1.0^{d}	0
E. coli	44.7 ± 0.6^{a}	42.0 ± 2.31^{b}	30.0 ± 2.3^{c}	18.01 ± 1.0^{d}	0

Table 3: Antimicrobial properties of GALWE using agar-well diffusion method. Zone Diameter Inhibition (mm)

Bacterial strain	Leaves extracts (g/ml)			Streptomycin	Negative control
	1	0.5	0.1	10 µg/disc	
S. aureus	35.7 ± 1.5^{a}	31.7 ± 1.2^{b}	18.7 ± 0.6^{c}	18.00 ± 1.0^{d}	0
E. coli	36.3 ± 1.2^{a}	33.7 ± 2.3^{a}	22.0 ± 2.3^{b}	18.01 ± 1.0^{c}	0

4. Conclusions

The fruits of *G. atroviridis* showed higher antimicrobial and antioxidant activities than leaves extracts.

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Antioxidant activity efficacy study of therapeutic flower extract of *Tagetes erecta* under different drying techniques

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Abstract
Therapeutic flowers have been regarded as significant functional culinary, medicines and cosmetics ingredients due to its diversified group of bioactive compounds contained in the epidermal cells, often reflected from the floral intensity. Drying is considered to be an important post-harvest step that limits the enzymatic degradation and prolongs the storage time. This study was aimed to evaluate the antioxidant activity of intense yellow flower extract of *Tagetes erecta* when subjected to freeze drying (FD) and multiple hot air convection oven drying (OD) conditions (at 50 °C for 1, 2, 3 and 4 hours, and 80 °C for one hour). The antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and Folin-Ciocalteu assay for total phenolic content (TPC). FD extract significantly exhibited the highest antioxidant activity compared to fresh flower and OD extracts with IC_{50} of 20 mg/L. Similarly, the highest TPC value of 99.33 mg of gallic acid equivalent (GAE) was observed in FD extract while OD 50 also showed increased in values with exposure time (9.54-21.97 mg GAE/g). These results demonstrated a strong correlation between DPPH free radical scavenging activity and TPC. Therefore, FD was shown as a highly promising drying technique for preserving antioxidant properties of *T. erecta*.

Keywords: *Tagetes erecta*, therapeutic flowers, bioactive compound, drying, antioxidant

1. Introduction

Plants in general have been traditionally used in many aspects of human life, including culinary, medicine and cosmetics due to their antioxidant and antimicrobial properties [1]. Various bioactive compounds contained in different plant parts such as leaves, roots, flowers, fruits and seeds are found to be vital in plant defence against pest and diseases [2]. These attributes were chemically analysed and an increasing number of bioactive compounds have been extracted and isolated, including the discovery of morphine and salicylic acid [3]. Flowers with intense colour are especially good indicators for antioxidant properties which correspond to the presence of a high concentration of flavonoids, anthocyanins and carotenoids. Similarly, flowers with lower floral intensity such as white are associated with lower antioxidant properties [4]. Floral intensity is also one of the most important post-harvest characteristics in cut flowers, where various drying methods have been employed to halt the enzymatic reactions that cause degradation and altered characteristics as well as prolongs the storage time [5]. However, the drying method itself might alter the bioactive compound characteristics since they are highly

sensitive to thermal degradation [6]. The objective of this study was to evaluate the antioxidant activity of flower extract of widely grown *Tagetes erecta* (marigold), which exhibited intense yellow colour, when subjected to freeze drying (FD) and multiple hot air convection oven drying (OD) conditions (at 50 °C for 1, 2, 3 and 4 hours, and 80 °C for one hour).

2. Materials and methods

2.1 Plant material

Fresh fully developed and undamaged flowers of *T. erecta* were collected from the district of Kluang, situated in the state of Johor in Malaysia. The flowers were carefully placed in plastics containers and covered with wet paper towels before being transported within an hour to Universiti Teknologi Malaysia (UTM), Pagoh for further processing. The petals were manually separated from the other parts (anther, stamen and sepals) and used for the experiments.

2.2 Moisture content analysis

The fresh petals were collected for the initial moisture content while samples were also taken after each treatment for residual moisture content in triplicate. The petals were distributed evenly onto trays and placed in a drying oven (Memmert GmbH, Germany) at 105±0.5 °C for 24 hours. Moisture content (Mw) was calculated based on a percent dry basis according to the following equation and the mean values were reported:

$$M_w = \frac{W_i - W_d}{W_d} \, x \, 100 \qquad (1)$$

Where, W_i was the initial mass of samples and and W_d was the mass of dried samples. In the case of samples taken after each treatment, W_d was the mass of samples after 24 hours of further drying.

2.3 Drying techniques

The fresh petals were dried following the methods described:

Freeze drying: The petals were distributed evenly onto trays and subjected to freezing and drying at -20 °C and 30 °C, respectively, in a freeze dryer (Cuddon, New Zealand) for 24 hours. The samples were then removed and immediately stored in a sealed plastic bags marked prior to extraction.

Low temperature convection drying: The petals were distributed evenly onto trays while avoiding overlapping. The trays were then placed in a drying oven (Memmert GmbH, Germany) at 50±0.5 °C for 1, 2, 3 or 4 hours, marked OD 50 1, OD 50 2, OD 50 3 and OD 50 4, respectively. The samples were removed from the oven at the set times and placed in a desiccator (PYREX, USA) to cool down to room temperature before being stored in sealed plastic bags prior to extraction.

High temperature convection drying: The petals were distributed evenly onto trays while avoiding overlapping. The trays were then placed in a drying oven (Memmert GmbH, Germany) at 80±0.5 °C for 1 hour, marked OD 80. The samples were then removed from

the oven, let cool to room temperature in a desiccator (PYREX, USA) and stored in sealed plastic bags prior to extraction.

2.4 Extraction procedure
The fresh, FD and OD samples were cut and crushed into relatively fine powders. Samples were than weighed and an immersed in methanol at a ratio of 1 g:10 ml filled in Falcon tubes. The tubes were then placed in a sonicator (Elma, Germany) at 30 °C for 10 minutes, after which they were removed and let to stand for 1 hour. Each content was filtered through a Whatman filter paper number 40 into another Falcon tube and stored at -20 °C prior to further analysis.

2.5 DPPH free radical scavenging assay
The free radical scavenging assay was carried out for all extracts in triplicate. DPPH solution was prepared in methanol and protected from direct sunlight. Ascorbic acid was used as standard. Serially diluted extracts were mixed with 100 µl of DPPH solution. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured at 515 nm using an ELISA reader (Molecular Devices, USA). The antioxidant activity was calculated as percent of inhibition (I) basis according to the following equation and the mean values were reported:

$$\text{Scavenging effect (\%)} = \frac{A_{DPPH} - A_{sample}}{A_{DPPH}} \times 100 \qquad (2)$$

Where, A_{DPPH} was the absorbance of the DPPH solution and A_{sample} referred to the absorbance when samples were present. IC_{50} was determined from the graph of percent scavenging effect against sample concentration (mg/L).

2.6 Determination of TPC
TPC of extract was determined in triplicate according to Folin-Ciocalteu (FC) colorimetric method using gallic acid as standard. Therefore, TPC was expressed as mg gallic acid equivalents (GAE)/g dry samples. Each extract was mixed with 100 µl of 10% FC reagent and let idle for 5 minutes, after which 80 µl of 7.5% aqueous sodium carbonate (Na_2CO_3) solution was added into the mixture. The mixture was then incubated at room temperature for 2 hours. The absorbance was measured at 750 nm using an ELISA reader (Molecular Devices, USA).

2.7 Statistical analysis
Data were analysed using IBM SPSS Statistics version 22. One-way analysis of variance (ANOVA) and Tukey's HSD ($p<0.05$) were performed to evaluate the significant differences between samples. The correlation between DPPH and TPC were evaluated by a multiple correlations test with Pearson's correlation coefficient. All values were expressed as means ± standard deviations (SD).

3. Results and discussion

3.1 Moisture content
The results of different drying techniques are shown in Table 1. The fresh flowers initially contained 90.00±0.50% of moisture. FD of *T. erecta* recorded the highest decrease in

moisture content (92.55%). OD 80 showed no significant difference compared to FD, with an average loss of 87.48%. There was no significant difference in OD 50 1 up to OD 50 3, where a significant difference was only observed in OD 50 4. These significant variations in residual moisture content were likely to affect the antioxidant properties, especially since there were also variations in colours retained. All OD 50 exhibited only a slight variation in colours, gradually turned darker with longer exposure. All OD samples exhibited apparent darker colours than the FD equivalents although there was relatively similar moisture content. These might be attributed to caramelization and the accumulation of melanoidins typically associated to thermal exposure [7].

Table 1: Residual moisture content from different drying techniques.

Conditions	Moisture content (%)
Freeze drying	6.71 ± 0.29^{a}
Oven drying (50 °C 1H)	88.67 ± 0.28^{b}
Oven drying (50 °C 2H)	87.30 ± 0.60^{b}
Oven drying (50 °C 3H)	80.29 ± 0.38^{b}
Oven drying (50 °C 4H)	71.54 ± 0.64^{c}
Oven drying (80 °C 1H)	11.11 ± 0.26^{a}

Different letters correspond to statistically significant ($p<0.05$).

3.2 DPPH free radical scavenging activities

Lower IC_{50} from lower absorbance of DPPH corresponds to higher antioxidant activity and vice versa. The effects of different drying techniques on DPPH scavenging activities are shown in Figure 1. FD of *T. erecta* (20 mg/L) significantly showed the highest antioxidant activity compared to the respective fresh (280 mg/L) and OD samples (180-560 mg/L). *T. erecta* was determined to be a very strong antioxidant. These variations in antioxidant activity recovery might be due to the presence of phenols and flavonoids which act as the free radical scavengers. Additionally, pigments that also act similarly might be a lesser factor since FD samples were visually less intense than OD samples but expressed higher antioxidant activity than the latter [8].

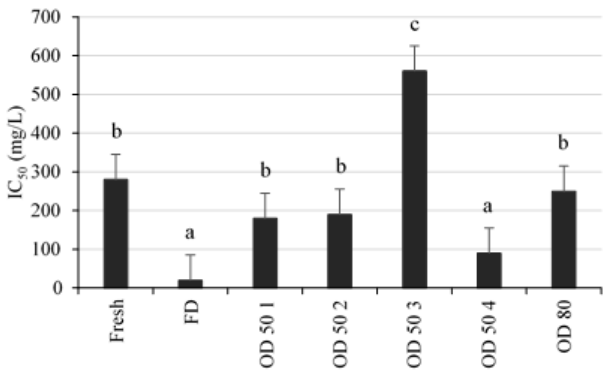

Figure 1: DPPH free radical scavenging activity recovery from various drying techniques. Different letters correspond to statistically significant ($p<0.05$).

3.3 Total phenolic content

TPC in various extracts were calculated using the linear regression equation of gallic acid ($y=0.0055x + 0.0696$, $R^2 = 0.9785$). The effects of different drying techniques on TPC are shown in Figure 2. FD of *T. erecta* showed the highest value of TPC (99.33 mg GAE/g) which was a significant recovery compared to fresh samples (5.10 mg GAE/g). The preservation of TPC have been found to significantly affected by thermal exposure, where longer exposure to temperatures ranging from 50-90 °C caused more degradation of TPC compared to shorter exposure at higher temperatures [9]. However, the apparent disagreement of the results to these findings might be explained by the sensitivity of the phenolic and flavonoid compounds [10]. This study has also showed that high antioxidant activity always corresponds to high values of TPC. Therefore, there was a strong correlation between the DPPH free radical scavenging activity and TPC, with R2 = 0.72 ($p<0.05$).

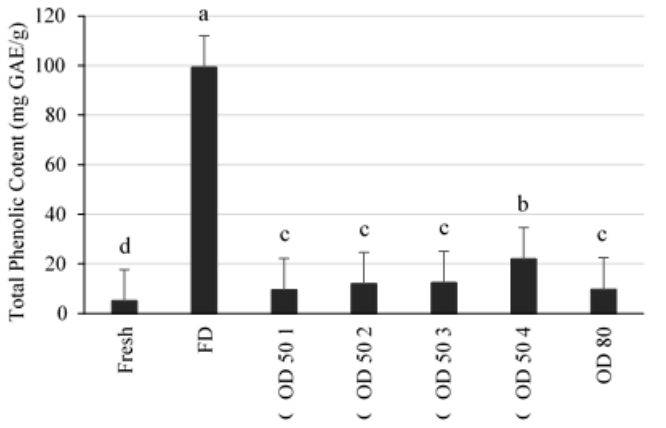

Figure 2: Total phenolic content recovery from various drying techniques. Different letters correspond to statistically significant ($p<0.05$).

4. Conclusions

This study showed the continuously close relation of floral intensity and bioactive compounds when subjected various drying techniques. Temperature, exposure time and residual moisture content can affect the preservation of antioxidant activities, which might then affect the quality of edible flowers especially when intended for consumption. Additionally, different species might respond differently to different drying techniques. Overall, FD was shown to be a highly potential alternative to other drying techniques, resulted in improved recovery of bioactive compounds. However, the complicated processes involved and long exposure time is likely to translate to high energy consumption which add to production costs. Therefore, the most suitable drying method for the intended quality and costs must always be taken into account.

Acknowledgments

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Growth development of pak choy (*Brassica rapa* L.) under drought stress when treated with plant growth consortium microorganisms (Pro-BacY) isolated from agrowaste

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Abstract
Drought is the major stress in this region which limits productivity. Microbial communities in the rhizosphere are of special importance to stress tolerance. The aims of this study to determine effects of plant growth consortium microorganisms named as Pro-BacY isolated from agrowaste on the growth performance of leafy vegetables grown in the non-sterile soil under drought stress. Therefore, pak choy or bok choy (*Brassica rapa* L. var. *chinensis*) is one of favorite green leafy vegetable was used as a plant model in this study. The soil was treated about 7 days with Pro-BacY before planted with pak choy under drought stress for 30 days cultivation. These results showed that the pots containing plants growth in untreated soil exhibited drought stress symptoms (leaf curling, wilting and senescence) after 7 days of planting. Then, after 10 days of cultivation start showing plant death. Although drought stress declined the growth of pak choy, these decreases were partly recovered by Pro-BacY inoculation in the soil. In addition, the microorganism's consortium associated with water and nutrients availability had a significant positive influence on soil organic matter, electro conductivity and pH of the soil, leaf number, total root length and total biomass of pak choy after 30 days of cultivation. Based on the results its showing increase about 57.22 %, 66.9 % and 65.35 % variations in leaf number, total root length and total biomass, respectively were attributable to the presence of certain soil microorganisms. In conclusion, this microbial communities study exhibited positive effects on the plant biomass, morphological and physiological parameters in leafy vegetables under drought stress via modification of the soil microbes for dryland agriculture.

Keywords: *Brassica rapa* L. var. *chinensis*, plant growth microorganisms, soil microbes, drought stress, plant performance

1. Introduction

Global climate change, including rising temperatures and disruptive weather patterns will affect ecosystem function, through a variety of direct and indirect ways. The climate change that causes environmental stresses such as drought which is major deterrents to plant growth responsible for decreased agricultural productivity. Drought affects plant water potential and turgor, enough to interfere with normal functions changing physiological and morphological traits in plants [1-2]. Furthermore, drought stress influences the availability and transport of soil nutrients, as nutrients are carried to the

roots by water. Drought stress therefore decreases nutrient diffusion and mass flow of water-soluble nutrients such as nitrate, sulfate, calcium, magnesium, and silicon [3]. Nevertheless, under drought stress the decrease in chlorophyll content was symptom of photooxidation [2-4]. Drought as a multi-dimensional stress, affects at various sub cellular compartment, cell organs and whole plant level [2-5]. Fresh weight and water content are common growth parameters that are affected by drought [6].

Beneficial microbes associated with plants are known to stimulate plant growth and enhance plant resistance to drought stresses. The plant growth-promoting microbes (PGRM) that are rhizobacteria and mycorrhizae, a key component of soil microbiota, could play vital roles in the maintenance of plant fitness and soil health under stressed environments [7-8]. The PGRM inhabiting rhizosphere and facilitating plant growth either through direct mechanisms which include production of phytohormones, enhanced availability of nutrients or by indirect mechanisms that include suppression of pathogens by antibiosis, synthesis of lytic enzymes and induced systemic resistance (ISR) [9]. Plant growth promotory activities of rhizobacteria/mycorrhizae have been reported during drought stress in maize, cucumber and mung bean [10-11]. Therefore, the aims of this study were to determine effects of plant growth consortium microorganisms named as Pro-BacY isolated from agrowaste on the growth performance of leafy vegetables grown in the non-sterile soil under drought stress.

2. Material and methods

2.1 Greenhouse experiments

For pot experiment, soil is collected, air-dried, sieved (2-mm/10-mesh) and analyzed for physical–chemical characteristics. Unsterile soil is clay, pH 4.1; 26 g dm−3 of organic matter. The correction is performed with nutrient inputs of NPK at 0.11 × 0.16 × 0.043 g L^{-1}, respectively, and the pH is adjusted with 2.9 g L^{-1} of lime before filling the pots. Twenty inoculated pak choy plants are sown in soil-filled pots treated with Pro-BacY and untreated soil. Pak choy plants age of 2 weeks are washed in sterile distilled water before transfer to soil pot. Control treatment is achieved by mixing the seeds with sterilized saline solution (0.85%). Three pak choy plants are kept in each pot (3 kg of soil per pot) for the total of 20 pots. The soil was treated about 7 days with Pro-BacY before planted with pak choy under drought stress for 30 days cultivation and untreated soil just watered every day with water.

The pots are arranged randomly with five repeats at ambient light and temperature, in a greenhouse. After the germination, to simulate a water stress, plants are left to grow with 30% of water field capacity. Evaluation is performed after thirty days of sowing. The leaf numbers, root length and biomass are determined.

3. Result and discussion

3.1 Growth characteristics of pak choy

Based on the results its showing increase about 57.22%, 66.9% and 65.35% variations in leaf number, total root length and total biomass, respectively were attributable to the presence of certain soil microorganisms (Table 1).

3.2 Comparison study of number of leaves of pak choy

These results showed that the pots containing plants growth in untreated soil exhibited drought stress symptoms (leaf curling, wilting and senescence) after 7 days of planting (Figure 1). After 10 days of cultivation, pak choy that growth in untreated soil start showing plant death (Figure 2). Although drought stress declined the growth of pak choy, these decreases were partly recovered by Pro-BacY inoculation in the soil. In addition, the microorganism's consortium associated with water and nutrients availability had a significant positive influence on soil organic matter, electro conductivity and pH of the soil, leaf number, total root length and total biomass of pak choy after 30 days of cultivation.

Table 1: The comparison study of growth characteristics of *pak choy* growth in soil inoculated with Pro-BacY and untreated soil for 30 days cultivation

Soil treatment	Growth parameter (average)		
	Number of leaves	Root length (cm)	Fresh weight (g)
Treatment with Pro-BacY	9.35±1.20[b]	10.81±2.03[a]	480±8.36[b]
Control	4.35±0.85[a]	3.57±0.08[a]	180.9±5.33[a]

Means with the same letters within a column are not significantly different at P<0.05. Fresh weight, number of leaves and root length were measured after 30 days cultivation.

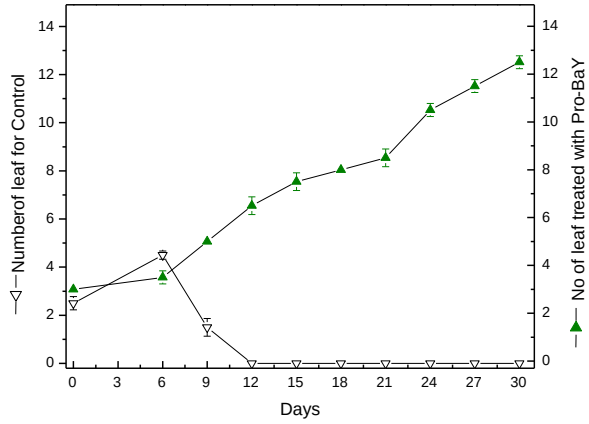

Figure 1: The comparison study of number of leaves of pak choy growth in soil inoculated with Pro-BacY and untreated soil for 30 days of cultivation

a) Control-untreated soil b) Soil treated with Pro-Bac(Y)

Figure 2: The growth of pak choy in untreated soil (a) and soil inoculated with Pro-BacY (b) and after 10 days of cultivation

4. Conclusion

Consortium of Pro-BacY give positive effects on the plant biomass, morphological and physiological parameters in leafy vegetables under drought stress via modification of the soil microbes for dryland agriculture.

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Effects of citric acid as natural preservative on the physicochemical stability of *Cocos nucifera* hand cream

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Abstract
Coconut (*Cocos nucifera*) oil is known for its potential and specifically beneficial for skin in reducing inflammation, keeping skin moisturized and helping heal wounds. Therefore, extensive development of cosmeceutical products incorporating *C. nucifera* oil had helped propel its global demand. The application of natural preservatives in skincare products is preferred to preserve therapeutic benefits of the bioactive compounds. In this study, citric acid was used as the natural preservative for the development of *C. nucifera* hand cream. The objectives of this study were to investigate and understand how the formulation affects the texture perception, through the study of the impact of the amount of (1) *C. nucifera* oil and (2) citric acid as the natural preservative. For that purposes, emulsions were developed by varying the amount of *C. nucifera* oil and citric acid concentration in a pH range of 3.77-7.95. The texture including pH, viscosity, spreadability, thermal stability and organoleptic characteristics were analysed, to develop a quantitative framework on the physiochemical characteristics of the *C. nucifera* hand cream. This study established consistent linear relationships between the concentration of *C. nucifera* oil and citric acid, and the physicochemical stability of the hand cream. This study confirmed the synergistic effects of *C. nucifera* oil and citric acid towards the physicochemical stability of the hand cream in elevating its cosmeceutical benefits.

Keywords: *Cocos nucifera*, natural preservative, bioactive compound, physicochemical stability, emulsion

1. Introduction

Coconut (*Cocos nucifera*) from the palm family (Arecacea) is an important tropical plant with many health benefits due to its wide array of bioactive compounds possessing antioxidant and antimicrobial properties [1]. These bioactive compounds can be found primarily in *C. nucifera* oil, of which the production has been steadily increasing to fulfil the demand for nutritional, pharmaceutical as well as cosmeceutical applications, specifically in skin care products [2]. *C. nucifera* oil is regarded as a safer and more efficient substitute for mineral oil which typically can be found in moisturizer products [3]. However, the use of *C. nucifera* oil in skin care formulation might interact with other ingredients which affect its bioactive compounds, necessitating the addition of preservative to guarantee consumer's safety [4]. Natural preservatives such as citric acid, has been shown to be a viable substitute to typical synthetic preservatives such as sodium benzoate, especially in preserving phenolic compounds associated with antioxidant properties [5]. Therefore, the objectives of this study were to investigate and understand

how the composition would affect the texture perception, through the study of the impact of the amount of (1) *C. nucifera* oil and (2) citric acid as the natural preservative on the physicochemical stability in terms of pH, viscosity, spreadability, thermal stability and organoleptic characteristics.

2. Materials and methods

2.1 Materials

Commercial materials for a basic formulation of hand cream were purchased from local retailers. Glycerine was used as humectant, aloe vera gel was used as moisturizing agent, citric acid was used as natural preservative and pH adjuster, while deionized water completed phase A of the formulation. Phase B comprised of ceteareth-25 as emulsifier, glyceryl stearate as stabilizer, cetyl alcohol as thickener, tocopherol as the source of vitamin E and refined *C. nucifera* oil as natural oil containing bioactive compounds.

2.2 Preparation of emulsions

Five emulsions were formulated with varying amount of refined *C. nucifera* oil (9.00-10.47 g w/w) and citric acid (0.03-0.60 g w/w), while one emulsion was formulated without citric acid and served as control. The water soluble ingredient in phase A were combined in a beaker with water, stirred and heated to 70 °C for 15 minutes on a hot plate (Stuart, UK). Similarly, oil soluble ingredients in phase B were combined in another beaker, stirred and heated for 15 minutes. Phase A was then added to phase B while still warm and immediately transferred to a homogenizer (IKA, Germany) for 10 minutes at 5000 rpm to form a homogeneous dispersion. The emulsions were cooled to room temperature and stored in airtight containers until further analysis.

2.3 Physicochemical evaluation of emulsions

2.3.1 pH

One gram of each emulsion was dispersed in 10 ml of deionized water and measured with a pH meter (Mettler Toledo, USA). The pH meter was calibrated with standard buffer solutions of pH 4,7 and 10 prior to each measurement. The measurements were performed in triplicate and the average values were reported.

2.3.2 Viscosity

A rheometer (Brookfield, USA) was used with spindle number 5 attached to determine the viscosity of each emulsion at 25 °C. All measurements were performed in triplicate and the average values were reported.

2.3.3 Spreadability

Spreadability of the emulsions was determined by keeping 1g of samples between two horizontal glass plates (10 cm × 20 cm). A standard 500 g of weight wad added to the upper glass plates and the spreading diameter was measured after 5 minutes. All measurements were performed in triplicate and the average values were reported.

2.3.4 Thermal stability

The emulsions were stored in airtight containers and incubated 25 °C and 37 °C for 48 hours to observe phase separation.

2.3.5 Organoleptic characteristics

The emulsions were characterized following the incubation period for physical appearance, colour, texture, phase separation and homogeneity by visual observation. A small quantity of samples was presses between the thumb and index finger for texture and homogeneity. Additionally, immediate skin sensation was also evaluated.

3. Results and discussion

The evaluation of pH, viscosity and spreadability were performed immediately after the preparation, while thermal stability and organoleptic characteristic evaluation were performed after the incubation periods.

3.1 pH

The pH values of the emulsions are shown in **Table 1**. The significant difference in formulated emulsions with citric acid confirmed its recognized capacity as pH adjuster in cosmeceuticals application. E1 (0.4% (w/w) citric acid) exhibited the lowest pH value while E5 (0.02% (w/w) citric acid exhibited the highest pH value. However, the values in E1, E2 and E3 (0.1-0.4% (w/w) citric acid) were insignificant compared to normal skin pH of 4.00-6.00.

Table 1: pH values of the emulsions immediately after the preparation

Emulsion	pH
Control	7.56
E1	3.77
E2	5.22
E3	6.13
E4	7.29
E5	7.95

Note: Percent compositions (w/w) (Control) 7.00% *C. nucifera* oil, (E1) 6.60% *C. nucifera* oil + 0.40% citric acid, (E2) 6.86% *C. nucifera* oil + 0.14% citric acid, (E3) 6.90% *C. nucifera* oil + 0.10% citric acid, (E4) 6.94% *C. nucifera* oil + 0.06% citric acid, and (E5) 6.98% *C. nucifera* oil + 0.02% citric acid.

3.2 Viscosity

The viscosity values of the emulsions are shown in Figure 1. Control, E4 and E4 exhibited relatively similar low viscosity, at 3 722, 4 672 and 2 972 cPs, respectively. E1, E2 and E3 exhibited relatively high viscosity, at 13 010, 15 550 and 12 740 cPs, respectively. This might be attributed to the protein behaviours in relation to pH, in which protein molecules might undergo an extensive denaturation in low pH condition thus increasing the viscosity or unfolded and expended at high pH condition causing lower viscosity [6].

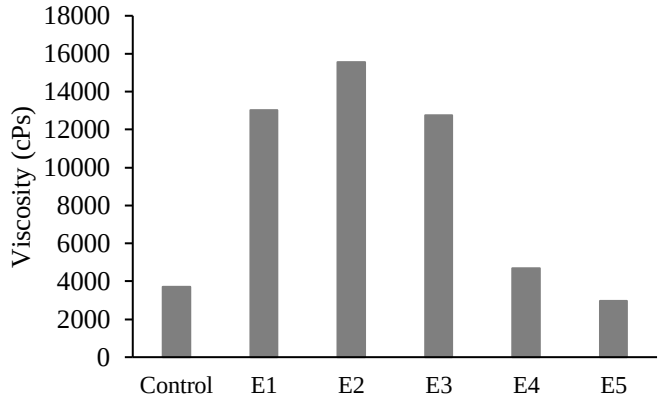

Figure 1: Effects of varying amount of *C. nucifera* oil and citric acid on the viscosity of the emulsions.

3.3 Spreadability

Figure 2 shows the spreading diameter of the emulsion after 5 minutes. The spreadability values were found to be in the range of 19.0-30.0 mm. E1 exhibited the lowest spreadibility (19.0 mm) while E5 exhibited the highest spreadability (30.0 mm). Lower spreadability might be attributed to lower presence of fatty acid due to lower amount of refined *C. nucifera* oil and vice versa. However, lower spreadability also means easier application on skin [7].

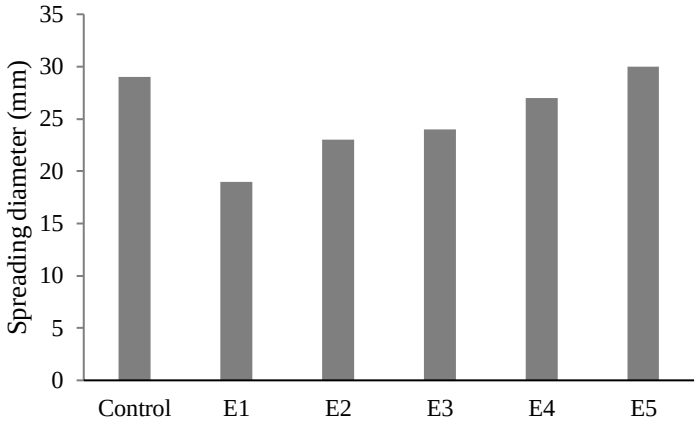

Figure 2: Spreadability behaviour of emulsions after 5 minutes.

3.4 Thermal stability and organoleptic characteristics

Physicochemical evaluation in terms of thermal stability and organoleptic characteristics of the emulsions is shown in Table 2. All emulsions appeared visually appealing with relatively similar physical appearance, colour and texture. No evident of phase separation was observed and homogeneity was cosmetically pleasing. It was on contact with skin that a slight variation was felt especially the smoothness of application owing primarily to the viscosity of the emulsions.

Table 2: Physicochemical evaluation of the emulsions

Emulsion	Physical appearance	Colour	Texture	Homogeneity	Phase separation	Immediate skin sensation
Control	Opaque	White	Smooth	Homogeneous	No	No grittiness, light, smooth
E1	Opaque	White	Smooth	Homogeneous	No	No grittiness, light, not greasy
E2	Opaque	White	Smooth	Homogeneous	No	No grittiness, smooth, not greasy
E3	Opaque	White	Smooth	Homogeneous	No	No grittiness, moisturizing, a bit thick
E4	Opaque	White	Smooth	Homogeneous	No	No grittiness, smooth, easily absorbed
E5	Opaque	White	Smooth	Homogeneous	No	No grittiness, light, smooth

4. Conclusions

This study confirmed the synergistic effects of *C. nucifera* oil and citric acid towards the physicochemical stability of the hand cream. Further study and optimization of formulation should elevate its cosmeceutical benefits.

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Antibacterial and antioxidant studies of freeze-dried *Momordica charantia* and green apple

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Abstract
Momordica charantia has been used as alternative medicine for diabetes, while green apple is known to contain high antioxidant and antimicrobial properties. The aim of this study was to investigate the antibacterial and antioxidant activities of freeze-dried *M. charantia* in combination of freeze-dried green apple. The combination was developed by incorporation of aqueous extract of freeze-dried *M. charantia* and green apple at the ratio of 1:1. Then, analysis of antibacterial and antioxidant were carried out. The results showed that incorporation of freeze-dried green apple extracts to freeze-dried *M. charantia* extracts contribute to a higher antibacterial and antioxidant properties. The addition of freeze-dried green apple extracts led to higher antibacterial properties of freeze-dried *M. charantia* extracts, if compared to the antibacterial properties of freeze-dried *M. charantia* extract solely. Besides, the combination of both extracts also resulted in higher antioxidant properties than the extract of freeze-dried *M. charantia* in the absence of freeze-dried green apple extracts. Thus, the combination of freeze-dried *M. charantia* and freeze-dried green apple extracts may be used as a functional drink with improved antibacterial and antioxidant properties.

Keywords: antibacterial, antioxidant, freeze-dried, *Momordica charantia*, green apple

1. Introduction

Momordica charantia or also known as bitter gourd or bitter melon, is a type of vegetable that belongs to Cucurbitaceae family and commonly used in many traditional medicinal recipes of Asia as well as other parts of the world [1]. It is also a well-known household medication for diabetes and may be used to stimulate lactation in breastfeeding mothers [2]. Charantin, the bioactive components that can be found in *M. charantia* plant was reported to provide therapeutic effect for diabetic patients [3]. Green apple, on the other hands, contains high antioxidant and antimicrobial properties [4-5]. According to reference [6], ascorbic acid is one of the organics acids found in green apple skin and one of the components responsible for antioxidant action. This bioactive compound is an abundant vitamin in apples [7]. Besides, the apple skin polyphenols also has been reported can provide protection against contamination by pathogenic microorganisms in foods [8]. However, there is no available reports on antibacterial and antioxidant activities of freeze-dried *M. charantia* and green apple. Thus, the objective of this study is to study the antibacterial and antioxidant activities of freeze-dried *M. charantia*, green apple and mixture of *M. charantia* plus bitter gourd extracts.

2. Materials and methods

2.1 Sample preparation
M. charantia and green apple fruits were washed and cut into thin slices. The seeds of both samples were discarded. The samples were put in trays and freeze-dried using Cuddon FD80 freeze-dryer. After that, the samples were collected, ground into powder and stored in -20 ºC freezer.

2.2 Preparation of M. charantia and green apple extracts
The extracts were prepared by weighing the weight of powder form samples (in milligram) and dissolved completely in one millilitre (1 ml) of distilled water. In this study, the concentration of 1000 ppm (1000 mg/L) for green apple (GA), *M. charantia* (bitter gourd, BG) and mixture of green apple plus *M. charantia* (GA+BG) samples with the ratio of 1:1 were prepared.

2.3 Antibacterial analysis

2.3.1 Bacterial isolates and collection
Two clinical bacterial isolates namely: *Escherichia coli* and *Staphylococcus aureus* were used in this study. Stock culture in an agar slant of these bacterial isolates were subjected to identification and confirmatory test.

2.3.2 Sub-culturing and purification of test organisms
Stock culture of the clinical bacterial isolates of *E. coli* and *S. aureus* were sub-cultured on nutrient agar and incubated at 37 °C for 24 hours. From the sub-cultured plate, a single of colony of each bacterial isolate was picked with a sterile inoculating loop and pure culture of these test organisms were obtained by streak plating method, then incubated at 37 °C for 24 hours. Pure culture each of these test organisms were used for the analysis.

2.3.3 Standardization of inoculum
A loopful of *E. coli* and *S. aureus* colonies from the pure culture plates were picked using sterile wire loop and emulsified in 9 ml sterile deionized water. Then, the turbidity was compared with 0.05 McFarland's Standard and measured by using UV/Vis Spectrophotometer at 600 nm.

2.3.4 Antibacterial susceptibility testing: agar well diffusion method
Nutrient agar plate surface was inoculated by spreading a volume of microbial inoculum over the entire surface. Then, a hole with diameter of 6 to 8 mm was punched aseptically with a sterile core borer. 50 µL of each extract was introduced into the well. Then, the agar plates were incubated in the incubator at 30 °C for 24 hours. Clear inhibition zones around the well denoting antimicrobial activity of the extracts were observed and measured. The diameter zones of inhibition (DZI) were measured in millimetre by using a meter ruler.

2.4 Antioxidant Analysis

2.4.1 DPPH assay
DPPH radical cation method [9] was modified to evaluate the antioxidant activity of *M. charantia* and green apple extracts. The DPPH reagent was DPPH (0.1 mM) dissolved

in 80% MeOH and ascorbic acid was used as positive control. To determine the scavenging activity, 100 µl of DPPH reagent was mixed with 100 µl of sample in a 96-well microplate and was incubated at room temperature for 30 minutes. After incubation, the absorbance was measured at 515 nm using a Versamax microplate reader. The IC_{50} DPPH values (the concentration of sample required for inhibition of 50% of DPPH radicals) were obtained through extrapolation from regression analysis. The antioxidant was evaluated based on this IC_{50} value.

2.4.2 ABTS assay

This assay was carried out following the protocol adopted in reference [10] with modifications. In this method, the ABTS radical was generated by reacting equal volume of 2.45 mM of $K_2S_2O_8$ and 7 mM ABTS solution prepared in water. The resulting solution was kept in the dark for 1218 hours at room temperature and further diluted with water in a ratio 1:50 until an absorbance of 0.700 ± 0.003 was attained at 734 nm. Equal volumes of extracts and ascorbic acid as positive control were reacted with $ABTS^+$ (1:1 v/v), left in the dark for 6 minutes and absorbance was taken at 734 nm. The half-inhibitory concentration (IC_{50}) of the extracts was computed from the graph of mean percentage ABTS inhibitory activity (taken in triplicates) against the equivalent of tested samples concentrations in linear regression.

2.5 Statistical analysis

All readings were taken in triplicates and analysed with GraphPad Prism8 statistical software. The mean values of tested samples were compared using Student's T-test. At $p<0.05$, means were considered significantly different and data were expressed as means ± standard deviation.

3. Results & discussions

3.1 Antibacterial studies

Table 1: Antibacterial effect after exposure of *Escherichia coli* and *Staphylococcus aureus* to sample extracts for 24 hours.

Test strains	Zone Inhibition (mm)			
	GA	**BG**	**GA + BG**	**Streptomycin (10 µg)**
EC	17.2500 ± 0.5000**	12.1250 ± 0.6292*	16.5000 ± 0.5774*	24
SA	17.1250 ± 0.2500**	11.5000 ± 0.5774*	15.2500± 0.5000*	

*Values are means of triplicate (n = 3) ± standard deviation where Streptomycin as positive control. GA: green apple, BG: bitter gourd, EC: Escherichia coli, SA: Staphylococcus aureus, ** P < 0.01, * P < 0.05.*

Results obtained in Table 1 indicated that all three extracts have higher antimicrobial activities against *E. coli* if compared to *S. aureus* bacteria, but the antimicrobial activity was not as high as the positive control. From the table, it can also be seen that GA extract had the highest inhibition zone (17.25 mm, 17.125 mm), BG extract showed the lowest inhibition zones (12.125 mm, 11.5 mm) while the combination of GA+BG extract showed

intermediate inhibition zones between GA and BG extracts (16.5 mm, 15.25 mm). This result showed that the addition of GA extracts to BG extracts has significantly increased the antimicrobial activity towards both test strains than the BG extract solely.

Previous study by reference [11] reported that tea and apple extracts containing phenolic compounds can significantly reduce the temperature required to kill foodborne organisms. Tea and apple skin extract also facilitated thermal destruction of *Escherichia coli* O157:H7 in cooked ground beef. Thus, this finding also supports the above antibacterial result that the addition of GA extract can improve the antibacterial properties of BG extract.

3.2 DPPH analysis

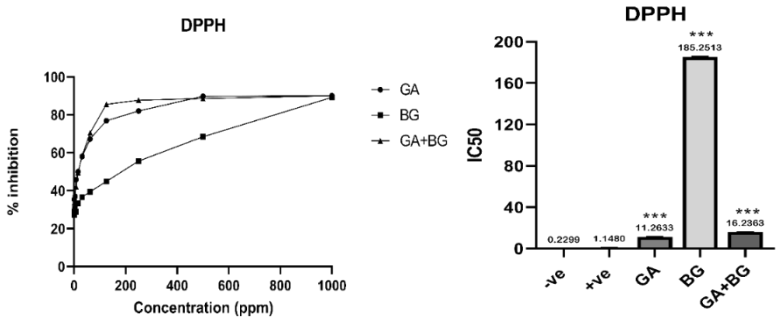

Figure 1: Values are means of triplicate (n = 3) ± standard deviation where ascorbic acid as positive control. GA: green apple, BG: bitter gourd, *** $P < 0.001$.

Antioxidant activity of natural crops and foods has been reported to possess an effect on numerous bioactivities such as whitening, anti-inflammation and high blood pressure. In antioxidant study, the DPPH assay offers a redox-functioned proton ion for unstable free radicals and plays an essential role in stabilizing destructive free radicals in human body [12]. The DPPH radical scavenging activity of tested samples revealed variations in scavenging capacity of extracts. Figure 1 provides the result of DPPH assay where diluted series of GA, BG and GA+BG extracts concentrations were used to estimate the concentrations at which fifty percent of DPPH radicals (IC_{50}) had been scavenged. The IC_{50} values of these extracts were then compared with ascorbic acid which is the positive control. A lower IC_{50} values indicates that the extracts possess a strong antioxidant activity. As showed in Figure 1, the highest antioxidant strength was observed in GA extract with the IC_{50} values 11.2633 mg/L followed by GA+BG extract with 16.2363 mg/L while the lowest antioxidant strength was recorded in BG extracts with IC_{50} values of 185.2513 mg/L. All extracts examined in this study were statistically significant when compared to the positive control and further prove that the combination of GA and BG extract showed a better and higher antioxidant activity to that of BG extract only.

3.3 ABTS analysis

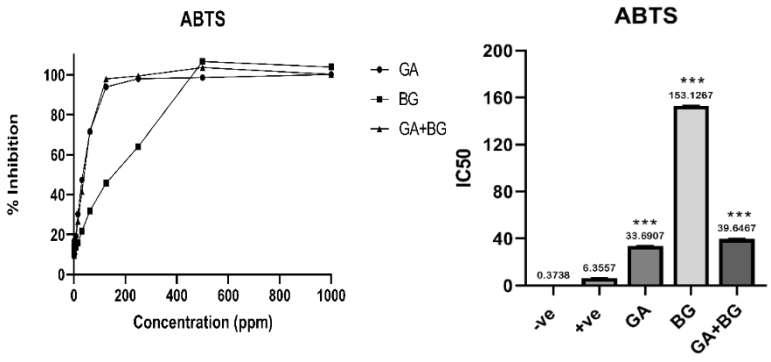

Figure 2: Values are means of triplicate (n = 3) ± standard deviation where ascorbic acid as positive control. GA: green apple, BG: bitter gourd, *** $P < 0.001$.

Results of percentage ABTS scavenging activities of extracts and positive control are presented in Figure 2. The IC_{50} values of the extracts were also compared with positive control which is the ascorbic acid. As visualized in Figure 2, the highest antioxidant strength was also observed in GA extract with the IC_{50} values 33.6907 mg/L followed by GA+BG extract with the values 39.6467 mg/L whereas BG extract was observed to show the lowest antioxidant activity with the IC_{50} values 153.1267 mg/L. All of these extracts were found statistically significant when compared to the positive control. This result also indicated that the most active extract was from GA extracts. It further suggested that the BG+GA extract was more active than BG extract solely. This result also was supported by reference [4] which stated that green apple cultivar contains more fibers, antioxidants, minerals, and vitamins than other cultivars of apple. Hence, consuming green apple is advisable for better human health [13].

4. Conclusion

The combination of green apple to *M. charantia* (bitter gourd) may have synergistic effects on its bioactivities. These include better antimicrobial protection against pathogenic microorganisms as well as nutritional and health benefits associated with the consumption of apple that are considered as natural antioxidants. Thus, the combination of both extracts may be used as a functional drink with improved antibacterial and antioxidant properties.

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