ISSN – 0215-1162

Cord

International Journal on Coconut R & D - Vol. 36, 2020

International Coconut Community

Cord

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Non-ICC Member Countries	**US$ 50.00**

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Integration of In Silico and In Vitro Approach to Reveal the Anticancer Efficacy of Virgin Coconut Oil

Babita Pruseth[1], Silvi Banerjee[1], Amit Ghosh[1*]

Abstract

Virgin coconut oil (VCO) has antioxidant properties and is being increasingly used as nutraceuticals and cosmeceuticals. It also has a long history of ethnopharmacological use. Anticancer effect of VCO has been reported in several articles. The main bottleneck of exploring the anticancer efficacy of VCO is the difficulty in identification and validation of target proteins and their regulated pathways. The work plan was in-silico analysis using Comparative Toxicogenomics Database (CTD) and STRING. CTD curated and integrated data for more than 5700 gene-disease and 2000 chemical-disease relationship. Medium Chain Fatty Acids (MCFAs) from VCO like Lauric acid, Caprylic Acid, Capric Acid, and Myristic acid can target almost 17 cancer-associated proteins. An attempt was made to identify the target proteins and their pathways regulated by VCO. We analyze curated and inferred VCO-gene expression data and illustrate the impact of VCO exposure on cancer-related gene network and molecular function. In enriched pathway analysis, it has been evident that all of them are the part of different cancer-associated pathways (Neoplasms, Digestive System Neoplasms, Urogenital Neoplasms, Liver Neoplasms). This response may mimic the biological response to VCO. In silico result was tested by in vitro study and VCO kill the Human hepatocellular carcinoma cell lines (hepG2). Based on the findings of this study and several published studies it is proposed that a VCO may have immense potential as a botanical product against cancer.

Key words: Virgin Coconut Oil, Liver, and Oral cancer, Protein targets, Pathway analysis, Human Intervention trial

Introduction

Anticancer effect of Virgin Coconut Oil (VCO) has been reported in several articles. A large number of the published article indicates the anticancer potential of VCO, especially in the colon, breast, lung, Liver and oral cavity. Lim-Sylianco (1987) published a 50-year literature review showing the anticancer effect of coconut oil **(1)**. Cohen *et al.* (1986) showed that coconut oil was far more protective than unsaturated oil in chemically induced colon and breast cancer. In his study, 32% corn oil eater developed colon cancer, as compared to only 3% of coconut oil eater **(2)**. Recently Law et al. (2014) reported that VCO consumption during chemotherapy helped improve functional status and global quality of life of breast cancer patients. It also reduces the symptom related to the side effect of chemotherapy **(3)**. MCFA compositions are altered in breast cancer tissue **(4)**. The lauric acid is a major component of the VCO and ranged from 46.36 – 48.42 %. Lauric acid-induced apoptosis in colon cancer cell by triggering oxidative stress **(5)**. Moreover, the anticancer activity of VCO against breast cancer cell line (SkBr-3) reported by Calderon *et al.* **(6)**. Yahaya *et al.* also reported the anticancer effect of VCO on lung cancer cells. VCO induced apoptosis in NCI-H1299 and A549 cell line **(7)**. Recently Enos et al. 2016 reported that the protective role of coconut oil in colon cancer induced by azoxymethane (AOM)/dextran sulfate sodium (DSS) in a murine model **(8)**. Famurewa *et al.* 2017 reported the antioxidant and hepatoprotective effects of VCO supplementation against hepatotoxicity and oxidative damage induced by anticancer drug methotrexate (MTX) in rats **(9)**.

Despite the diverse and serious beneficial effect of VCO, its molecular mechanism of action yet to be identified. To translate, the most relevant missing data are to identify the target protein and pathways, followed by human intervention trials. To identify target protein and pathway associated with the anticancer effect of VCO, Comparative Toxicogenomics Database (CTD) and STRING was used. In

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addition to that anticancer efficacy of VCO and Fractionate coconut oil was tested in liver cancer cell line (HepG2).

Method

Curation process (VCO-gene-disease interaction)

CTD provides the information about VCO-gene-Disease relationship by integrating data curated from the scientific literature. Candidate references for curation in CTD were collected by querying PubMed for co-occurrence of associated disease and chemical name. The fatty acid composition of VCO range from C8-C18 and predominant MCFAs are Lauric Acid, Caprylic Acid, Capric Acid, and Myristic Acid. The fatty acid name was used as a query term to retrieve the top 10 associated diseases. Each compound associated disease and corresponding gene expression signature was evaluated against a pre-existing literature to predict they're possible anticancer efficacy. The analysis reported here was based on data downloaded and analyzed in July 2018.

Compound Signature Matching: All chemical-disease links known as a marker or therapeutic agent in CTD. MCFAs of VCO induced gene expression signatures were evaluated against pre-existing disease-associated gene signature library in order to make a prediction of their anticancer efficacy. Compounds linked to most relevant disease were explored using CTD. However, in order to reduce the noise and to focus on the most relevant information, only the top 5 associated diseases with each compound were considered for further study. Among these only chemical – cancer data with CTD interference score above 5 were considered for the chemical-disease association.

Disease analysis: Curated and inferred interaction VCO MCFAs-gene were combined. Total 17 genes, selected by compound-disease-gene interaction were considered for further study. Among these genes, a number of genes overlapped with major VCO compounds. Total 40 genes interact with MCFAs of VCO. These data indicate that major VCO compounds share some common molecular activity, although the overall biological effect or the molecular network they invoke may be compound specific. This approach may reasonably mimic a biological response to VCO. CTD set analyzer tool was used to demonstrate the combined gene network as well as disease and pathway enrichment.

Gene Ontology enrichment analysis: The GO is an independent annotation resource used by bio curators to characterize gene product's molecular function (GO-MF), the Cellular compartment (GO-CC), and Biological process (GO-BP). The analysis was derived from data available in CTD in July 2018. GO-CC query term included "plasma membrane protein", "nucleus" and "intracellular compartment" etc.

Protein-protein interaction: Predicted protein-protein interactions (PPI) were obtained from the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database. PPI network is a useful tool to decipher the mechanism of action. In PPI, nodes represent protein and edges represent interaction ie. the binding of one protein to another. These proteins were analyzed by STRING database to create interacting proteins network.

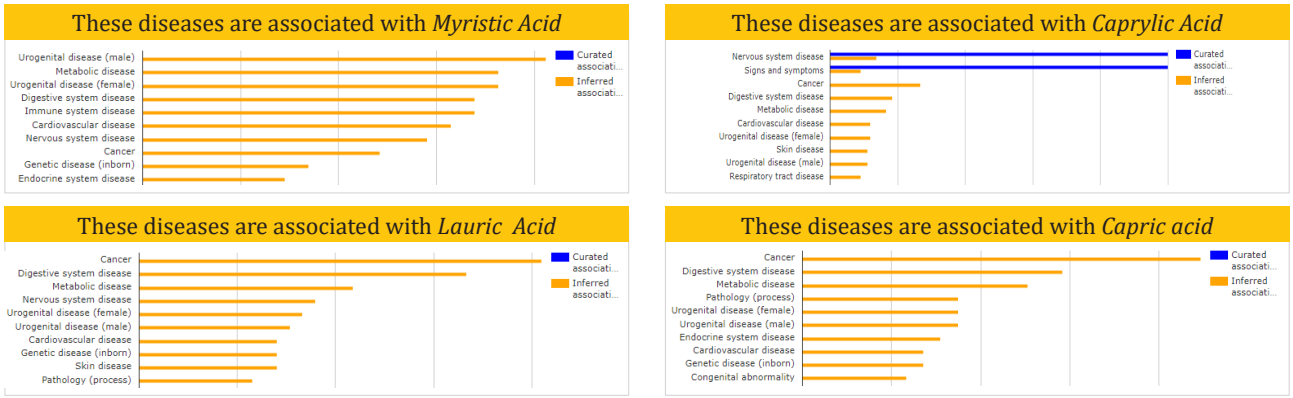
Co-expression: Co-expression analysis relies on the hypothesis that highly correlated gene are biologically related. Co-expression network analysis utilizes entire measured transcription to help to determine gene function and mode of action.

Cell Culture: This study is a cell culture-based study. The study was conducted by using HepG2 (hepatocyte carcinoma cell line) cell line. Cells were obtained from the National Center for Cell Science, Pune (NCCS), India. All the cell cultures were performed in a class two laminar flow cabinet and the cells were maintained at 37˚C in a humidified atmosphere of 5% CO2 and 95% air under aseptic condition. Chemicals were purchased from different known companies. A standard protocol was followed for the above-mentioned assay.

Treatment of Cells: Cell line was treated with different concentrations of Virgin Coconut Oil (VCO), and Fractionated Coconut Oil (FCO). Cells were harvested after 72hr of incubation.

Determination of Cytotoxicity and Cell Viability by MTT Assay: Cells were seeded into 35mm culture dishes containing suitable growth media and were maintained at 37˚C in a humidified atmosphere of 5% CO2 and 95% air under aseptic condition. After 24 hours, cells were treated with different concentrations of VCO and Fractionated coconut oil (FCO). FCO contain C8:0-54.15%; C10:0-45.11%. Dose-response was verified using 3-(4,5-Dimethylthiazol-2-

Figure 1. Showing the top 10 diseases associated with SCFAs of VCO (X axis represents the types of diseases associated with each fatty acids)



yl)-2,5-diphenyltetrazolium bromide (MTT) analysis. Each treatment was repeated 3 times. The cells were then incubated with MTT in the growth medium for 4h at 37°C. Cell viability was evaluated by comparison with a control culture (assumed to be 100% viable). VCO and FCO dose and the incubation period were standardized on the basis of a previous study.

Statistical Analysis: The whole procedure was repeated three times. The results were presented as mean ± Standard Error of the Mean (SEM). Significant changes were assessed by Student's t-test. A value of P <0.05 was considered significant.

Result

To determine whether VCO affect cancer-associated gene, we explore cancer-associated gene set interacting with different MCFAs of VCO. The major SCFAs of VCO is Lauric acid, Caprylic acid, Capric Acid, and Myristic acid predominantly associated with the cancer-associated pathway (Fig. 1). The top 5 associated diseases with each compound of VCO were selected based on their inference score, which shows the degree of similarity between CTD chemical–gene–disease networks and a similar scale-free random network and subjected to further study.

Four components interact with 17 cancer-associated genes (ALB, ATF3, CAV1, CLDN1, CXCL8. CYP2E1, CYP4B1, MAPK14, NR1H2, POR, PPARA, PPARG, PPARGC1A, RELA, TTR, UGT2B7) which are associated with a neoplasm (digestive system, liver, lung, urogenital etc.). The disease was subdivided into a specific type of malignancies. (Table 1).

Pathway analysis showed that the network was enriched with the role in Neoplasms (*alb*, *atf3*, *cav1*, *cldn1*, *cxcl8*, *cyp2c9*, *cyp2e1*, *cyp4b*, *mapk14*, *nr1h2*, *por*, *ppara*, *pparg*, *ppargc1a*, *rela*, *ttr*, *ugt2b7*), Digestive System Neoplasms (alb, atf3, cav1, cxcl8, cyp2e1, mapk14, nr1h2, ppara, pparg, rela), Urogenital Neoplasms (atf3, cav1, cxcl8, cyp2e1, cyp4b1, por, ppara, rela, ugt2b7), Liver Neoplasms (atf3, cxcl8, cyp2e1, mapk14, nr1h2, ppara, pparg).

GO pathway analysis shows most of gene product location is intracellular and gene products are the transcription factor and have DNA, RNA, and protein binding activity (Table 2).

The anticancer implication of VCO- genes network is consistent with KEGG pathway and Disease association. CTD based analysis provide candidate gene that may help to explain the underlying mechanism of action of VCO.

We illustrate the PPI network of the candidate genes, which revealed the confirmed interaction of the following protein proved by experiment and from a curated database. Besides this inferred protein-protein interaction also revealed based on protein homology, gene neighbourhood, text mining, and protein homology. Most of this protein co-expressed in human and other species. Pathway analysis from protein-protein interaction (STRING) also reveals that cancer-related pathways are associated with candidate genes (Fig 2).

As mentioned above we have investigated the effect of VCO by adding the DMSO as a solvent in 1:5 ratio. The vehicle control (DMEM and DMSO in 5:1 ratio) was also used. In the case of

Disease Name (Top 10 out of 27)	Annotated Genes Quantity	Annotated Genes
Neoplasms	16	ALB|ATF3|CAV1|CLDN1|CXCL8|CYP2E1|CYP4B1|MAPK14|NR1H2|POR|PPARA|PPARG|PPARGC1A|RELA|TTR|UGT2B7
Neoplasms by Site	15	ALB|ATF3|CAV1|CLDN1|CXCL8|CYP2E1|CYP4B1|MAPK14|NR1H2|POR|PPARA|PPARG|RELA|TTR|UGT2B7
Digestive System Neoplasms	10	ALB|ATF3|CAV1|CXCL8|CYP2E1|MAPK14|NR1H2|PPARA|PPARG|RELA
Urogenital Neoplasms	9	ATF3|CAV1|CXCL8|CYP2E1|CYP4B1|POR|PPARA|RELA|UGT2B7
Liver Neoplasms	7	ATF3|CXCL8|CYP2E1|MAPK14|NR1H2|PPARA|PPARG
Neoplasms by Histologic Type	10	ALB|CXCL8|CYP2E1|MAPK14|NR1H2|POR|PPARA|PPARG|PPARGC1A|RELA
Neoplasms, Glandular and Epithelial	9	ALB|CXCL8|CYP2E1|NR1H2|POR|PPARA|PPARG|PPARGC1A|RELA
Lung Neoplasms	7	ALB|ATF3|CAV1|CXCL8|CYP2E1|MAPK14|TTR
Respiratory Tract Neoplasms	7	ALB|ATF3|CAV1|CXCL8|CYP2E1|MAPK14|TTR
Thoracic Neoplasms	7	ALB|ATF3|CAV1|CXCL8|CYP2E1|MAPK14|TTR

Table 1. cancer-associated genes associated with different cancer (CTD)

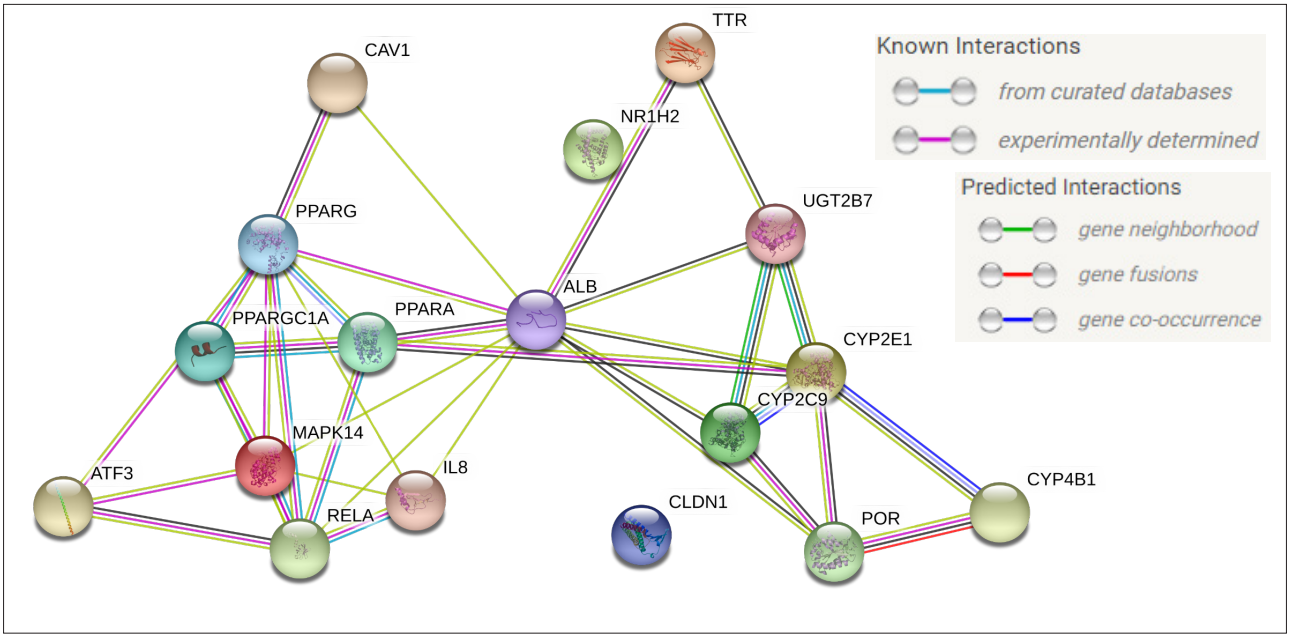


Figure 2. Interaction of protein which expression is modulated by VCO

GO Molecular Function (top 10 out of 21)	Gene
enzyme binding	9
organic cyclic compound binding	12
transcription factor binding	6
heterocyclic compound binding	11
oxygen binding	3
RNA polymerase II proximal promoter sequence-specific	5
DNA binding	14
protein binding	5
proximal promoter sequence-specific DNA binding	3
nuclear receptor activity transcription factor activity, direct ligand-regulated sequence-specific DNA binding	3

GO Biological Process (top 10 out of 218)	Gene
monocarboxylic acid metabolic process	9
fatty acid metabolic process	8
response to drug	10
cellular response to chemical stimulus	13
response to chemical	14
small molecule metabolic process	11
response to lipid	9
lipid metabolic process	10
carboxylic acid metabolic process	9
cellular response to lipid	8

GO Cellular compartment (top 10 out of 218)	Gene
intracellular	16
intracellular organelle part	14
organelle part	14
intracellular membrane-bounded organelle	14
cell part	16
cell	16
membrane-bounded organelle	14
intracellular part	15
intracellular organelle	14

KEGG Pathway (top 10 out of 218)	Gene
Hepatitis C	5
Insulin resistance	4
Non-alcoholic fatty liver disease (NAFLD)	4
Shigellosis	3
Epithelial cell signaling in Helicobacter pylori infection	3
Adipocytokine signaling pathway	3
RIG-I-like receptor signaling pathway	3
Pertussis	3
Salmonella infection	3
Longevity regulating pathway	3

Enriched diseases (top 10 out of 111)	Gene
Neoplasms	16
Neoplasms by Site	15
Female Urogenital Diseases	12
Female Urogenital Diseases and Pregnancy Complications	12
Male Urogenital Diseases Endocrine System Diseases	12
Nutritional and Metabolic Diseases	10
Digestive System Neoplasms	11
Liver Diseases	10
Diabetes Mellitus, Experimental	11

Table 2. GO and KEGG annotation for VCO SCFAs interacting gene

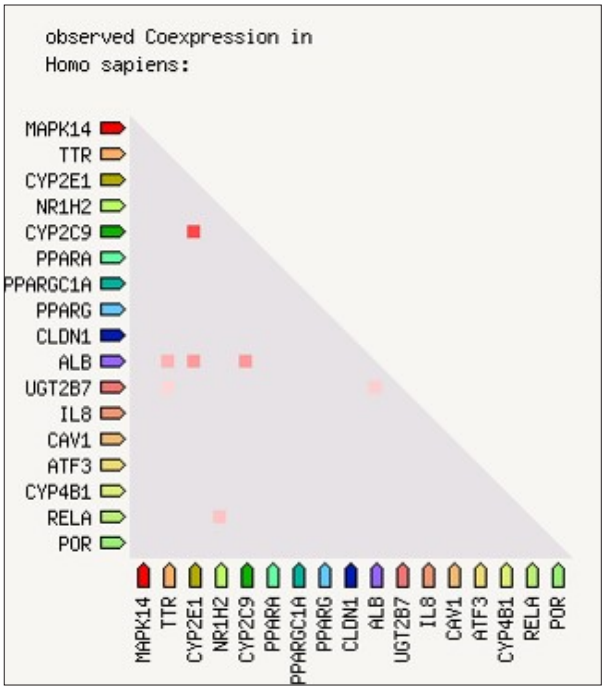


Figure 3. Showing the co-expression of VCO – cancer-associated genes

HepG2 cells, the VCO along with solvent showing the significant cell death with 20%, 40%, 60%, and 80% when compared to control (Fig. 4). We observed the similar results when the same cell lines were treated with Fractionated coconut oil (FCO) along with solvent (Fig. 5). All experiments were repeated three times.

Phase contrast images of control and treated with 20% of FCO or VCO with a solvent in the HepG2 cell lines are given below (Fig. 4 and 5).

The result of the in-vitro cell culture-based study clearly indicates that VCO may have some anticancer component which is also present in FCO.

The fatty acid may be the MCFAs. As in all VCO, amount of Lauric acid range from 46%-48%, so we have interested about in-silico analysis of the possible anticancer role of Lauric acid along with other fatty acids in VCO.

Discussion

To determine whether different fatty acid of VCO regulate a cancer-associated gene, we explore the interacting gene set with neoplasm associated gene set. VCO-disease relationship is clearly labelled as either the direct or inferred. A direct relationship is explored by integration of gene-disease relationship from the OMIM database. The inferred relationship is established by integrating curated gene-disease data. It was also found that SCFAs of VCO can target almost 17 cancer-associated proteins. Almost 50% of VCO is Lauric Acid which interacts with 18 genes and associated with several diseases among which cancer, digestive disease, Metabolic Disease, Nervous System Disease, and Urogenital Diseases are top five. Caprylic Acid interacts with 14 genes and associated with several diseases- Nervous System Disease, Sign and Symptoms, cancer,

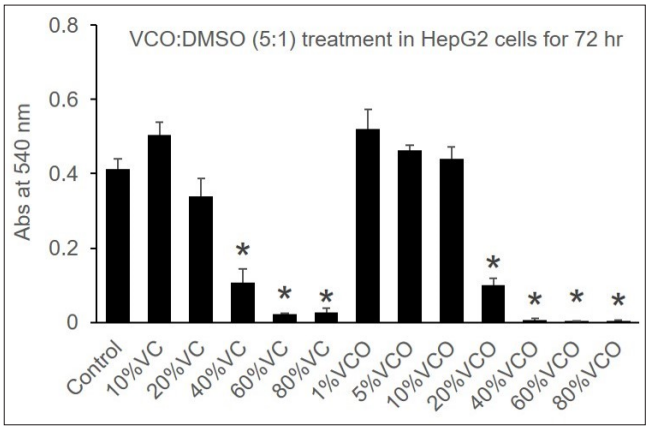


Figure 4. Cell viability assay after treatment with various concentrations of VCO and DMSO (in 5:1 ratio) in HepG2 cells for 72 hr. Student's-t test was performed to analyze the significant difference between the control and VCO treated cells. Mean ± SEM. *p ≤ 0.05

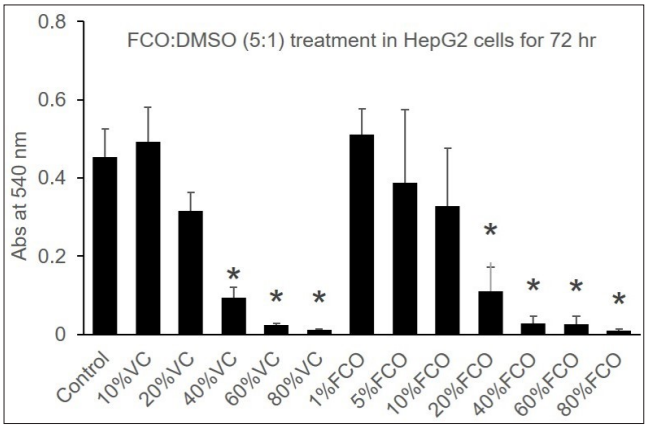
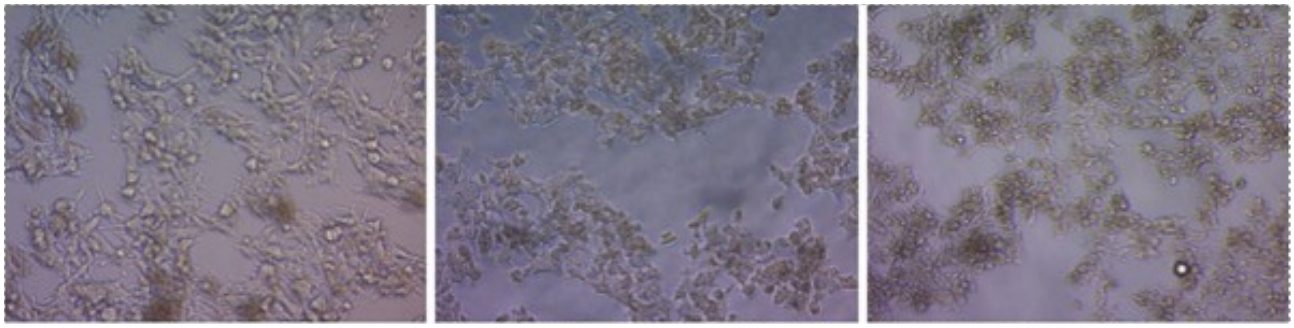


Figure 5. Cell viability assay after treatment with various concentrations of FCO and DMSO (in 5:1 ratio) in HepG2 cells for 72 hr. Student's-t test was performed to analyze the significant difference between the control and VCO treated cells. Mean ± SEM. *p ≤ 0.05



Control-HepG2 cells 20% VCO treatment in HepG2 cells 20% FCO treatment in HepG2 cells

Figure 6. Phase contrast images (20x) of HepG2 and KB cells after treatment with 20% VCO: DMSO (1:5) and FCO: DMSO (1:5)

digestive disease, and Metabolic Disease are top five. Capric acid also interacts with 8 genes and top five associated diseases are Cancer, Digestive disease, Metabolic Disease, Nervous System Disease, and Urogenital Diseases. Remarkably top five associated disease of 3 MCFAs of VCO are same. In case of Lauric acid 10 out of 18 genes, Caprylic acid 8 out of 14 genes and Capric acid 6 out of 8 genes associated with Cancer.

CTD analysis indicated that almost all of 17 proteins identified are the part of the cancer pathway and apoptosis. VCO regulated the expression of several genes. The top 10 Genes has been associated with selected pathway and disease association, indicating VCO may have modulate disease pathology of cancer (Table 1). Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway enrichment analysis shows to 10 pathways shows their associated with cancer (Table 2). KEGG is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from genomic and molecular-level information. These target genes were involved with many pathways and related disease like, Neoplasms, Urogenital Diseases, Digestive System Neoplasms, metabolic Disorder etc. The Gene Ontology (GO) analysis study was predicted the top 10 associated biological processes, cellular components and molecular functions related to the given set of targets gene that are regulated by VCO. The GO describes the biological domain with respect to three aspects: Molecular Function, biological process and cellular components. In enriched pathway, KEGG analysis came up with the diagram of cancer-associated pathways and all of them are the part of different cancer-associated pathways. The list of the VCO target genes (proteins) was subjected to STRING (Version: 11.0) analysis to reveal functional interactions between the VCO target proteins (Fig 2). Each node represents a protein, and each edge represents an interaction. STRING analysis was used to visualize the relationship amongst VCO target genes and Fig 3 shows the co-expression of target genes.

Reportedly MCFAs of VCO can be targeted to the liver and oral cavity. So to test the in silcio results, the effect of VCO was tested in on HepG2 liver cancer cells. Cell viability assay was done and a statistically significant minimum dose of 20% VCO was found to be effective against HepG2 liver cancer cells and 40% VCO almost kill the entire liver cell. As per human protein atlas out of 17 genes, the *alb*, *ttr*, *rela*, *ppara*, *mapk14*, and *por* RNA expression are significantly high in HepG 2 cell followed by medium level RNA expression of *ugt2b7*, *cyp2e1*, *ppargc1a*, *atf3*, *pparg*, *nr1h2*, and *cldn1*. As per in silico result, above mention gene are associated with liver neoplasm. In cell culture experiment, the effect of VCO on HepG2 cell also support the anticancer efficacy of VCO. In this experimental and in-silico analysis along with previously published result indicate VCO may have potent anticancer efficacy. However, the most relevant data that would support a protective effect of VCO on the treatment of Liver cancer are human intervention trials. A multi-centric, cost-effective trial may be initiated to test the therapeutic efficacy of VCO against liver neoplasm.

Today, drug discovery against cancer is one of the goals frequently pursued around the world. Natural products from plants are the endless source of new pharmaceuticals. Till now, three botanical drugs have been approved by FDA. *Sinecatechins* (VEREGEN®) [(15)] was the first botanical drug approved in 2006. The second drug is *Crofelemer* (FULYZAQ™) [(16)], approved by FDA in 2012 and the third drug is GRASTEK® [(17)], approved

in 2017. Botanical drugs have quick and large market acceptance and are quite a financial success as well. To upgrade VCO from potential anti-cancer agent to the status of 'Botanical Drug' human intervention trial is essential. A quick review of the published papers indicates that until now no human intervention trial was conducted with VCO as a preventive drug against liver cancer. Use of VCO for therapeutic purpose as a drug will enhance its value and economically benefit coconut industry.

Drawback: The interesting in silico result is supported by cell culture-based study. Though the outcome is very interesting, but it is a preliminary result. More detail study and clinical trial is required to establish the anticancer potential of VCO.

Conclusion: The outcome of this preliminary study indicates the anticancer potential of VCO.

Several studies indicate the anticancer effect of Virgin coconut oil (VCO) especially in the colon, breast, lung, Liver and oral cavity. Despite the diverse and serious beneficial effect of VCO, its molecular mechanism of action yet to be identified. The present study was attempt to identify the target proteins and their pathways regulated by VCO through in silico approach using Comparative Toxicogenomic Database (CTD) and STRING and again the in silico result was examined by in vitro study to test the anticancer efficacy of VCO on liver cancer cell line (HepG2). CTD set analyzer tool was used to demonstrate the combined gene network as well as disease and functional enrichment. The fatty acid composition of VCO range from C8-C18 and predominant MCFAs are Lauric Acid, Caprylic Acid, Capric Acid, and Myristic Acid were exposed to CTD to identify VCO-gene-Disease relationship, from where the top 5 associated diseases with each compound of VCO were picked out by the chemical-disease interaction and a number of 17 candidate genes were selected by the compound-disease-gene interaction. Pathway enrichment analysis indicated that almost all of 17 proteins identified are the part of the cancer pathway and apoptosis, where the GO analysis showed that most of gene product location is intracellular and gene products are the transcription factor and have DNA, RNA, and protein binding activity and STRING analysis revealed the functional interactions between the VCO target proteins. Further the in-silico result was extended to cell culture experiment to test the effect of VCO on HepG2 liver cancer cells, where VCO was found to be effective against HepG2 liver cancer cells and kill the entire liver cell. As per human protein atlas out of 17 genes, the *alb*, *ttr*, *rela*, *ppara*, *mapk14*, and *por* RNA expression are significantly high in HepG 2 cell followed by medium level RNA expression of *ugt2b7*, *cyp2e1*, *ppargc1a*, *atf3*, *pparg*, *nr1h2*, and *cldn1*. Hence it was proved the association of the 17 genes with liver neoplasm and from the cell culture study, the effect of VCO on HepG2 cell also support the anticancer efficacy of VCO. Thus, the present study along with previously published result indicate VCO may have potent anticancer efficacy, which could serve as useful sources for new anticancer agents for the treatment of Liver cancer.

Conflict of Interest

There are no conflicts of interest.

Acknowledgment

This work has been supported by AIIMS Bhubaneswar.

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Detection of Weligama Coconut Leaf Wilt Disease Phytoplasma by Real-Time Polymerase Chain Reaction

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Abstract

Weligama Coconut Leaf Wilt Disease (WCLWD) is a non-lethal, but debilitating phytoplasma disease found in coconut palms in Sri Lanka which is confined to the Southern Province of the country, well-away from the major coconut growing area. If it spreads to the major coconut growing area, it might severely damage the coconut industry in Sri Lanka. Government commenced a disease control program to eradicate the disease and, more importantly to prevent spreading of the disease to major coconut growing areas. The major constraint in this program is the lack of an accurate and reliable method for identifying affected palms. Visual symptoms are used to identify the affected palms for removal, yet growers are not always convinced of the method of resisting palm removal. This poses a serious threat to the implementation of the disease control program. Although a Nested-PCR-based disease diagnosis was established earlier, the detection rate and reliability need further improvements. Therefore, an urgent necessity for a more reliable disease detection method has arisen. In the current study, a Real-Time Polymerase Chain Reaction (qPCR) powered by a pair of primers and a probe designed from the published partial sequences of the WCLWD phytoplasma was validated with 202 coconut samples and a detection rate of above 95% was achieved. This newly established detection system was highly reliable and a way forward for controlling the WCLWD disease in Sri Lanka.

Key words: Coconut, weligama coconut leaf wilt disease, phytoplasma detection, qPCR

Introduction

Weligama Coconut Leaf Wilt Disease (WCLWD) was first reported in the Weligama area of the Matara district of Sri Lanka in late 2006. Later it was found that WCLWD has spread to all coconut growing Divisional Secretariat (DS) regions in Matara and four DS regions each in Galle and Hambantota districts in Southern Province (Wijesekara *et al*., 2008). WCLWD is a debilitating phytoplasma disease of coconut which poses an alarming threat to coconut industry in Sri Lanka. In the survey conducted by Coconut Cultivation Board (CCB) the yield loss due to WCLWD has been estimated as 18%, 2.42% and 25.87% in Galle, Hambantota and Matara districts, respectively. The reduction in kernel weight of severely WCLWD affected and leaf rot affected palms were 40% and 70%, respectively (Nainanayaka and Ranasinghe 2013).

The government has decided to remove the affected coconut palms to prevent its spread to major coconut growing area, termed as "the coconut triangle" which included 75% of the total coconut growing areas the country. Identification of affected palms was mainly based on the three prominent visual symptoms in the crown; flaccidity of leaf blade, uneven yellowing of fronds in the crown and marginal necrosis of leaflets. Bud leaves of some of the WCLWD affected palms were prone to be infected by a complex of fungi causing leaf rot disease as the final stage of the disease (Wijesekara *et al*., 2008;

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Wijesekara and Fernando 2013). Since yellowing of coconut fronds occurs due to various reasons, such as nutrient deficiencies, moisture deficit, *etc*., the identification of affected palms by visual symptoms alone is challenging.

During the palm removal program, some growers disagree with removing diseased palms because some of the marked palms are unaffected by the disease but the symptoms are due to some other reasons. In 2012, a Nested PCR based detection system was developed for the identification of diseased palms (Perera *et al*., 2012). However, the detection percentage of the palms showing visual symptoms of WCLWD by Nested PCR system was around 85% during rainy season. This level of accuracy was found to be inadequate for the reliable detection of suspected WCLWD palms (Wijesekara *et al*., 2013). Many workers have identified PCR based method can be used in routine identification of phytoplasma infections in several crops including tree crops (Deng and Hiruki 1990, Gundesen and Lee 1996). Therefore, a more reliable diagnostic technique for detecting WCLWD affected palms aroused and the use of Real Time Polymerase Chain Reaction (qPCR) was investigated.

Method and Materials

Milky white bud leaf samples of WCLWD affected palms were collected from different lands of disease-affected areas. Healthy samples were collected from Isolated Seed Garden (ISG), Ambakelle and Pallama Seed Gardens situated in North Western Province far away from the disease affected area where disease has never been reported. A total of 202 affected and 70 healthy coconut samples were collected for the experiment during the period of two years, from 2013 and 2014. DNA from white leaf disease affected sugarcane plants was used as positive controls to check the PCR reactions.

Bud leaf samples were surface sterilized with 70% alcohol and mid rib of the leaflets (ekel) were collected for the DNA extraction. Ekel was cut into small pieces and ground to a fine powder in liquid nitrogen. Total DNA was extracted by CTAB method (Sambrook *et al*., 1982) with slight modification (Perera *et al*., 1999). Composition of the extraction buffer was 0.05 moles Tris, 0.01 moles EDTA, 0.7 moles NaCl, CTAB 10 g and final volume 500 ml at pH 8; 2% monothioglycerol was added just before use. One gram of ekel powder was added into 2 ml Eppendorf tube with 1 ml of extraction buffer at 65 0C. The powdered ekal was mixed well with buffer and incubated at 65 0C for 30 minutes thoroughly mixing at 10-minute intervals. Tubes were allowed to cool to room temperature and 1 ml of chloroform: isoamyl alcohol (24:1) mixture was added and thoroughly mixed. Tubes were centrifuged at 13000 rpm for 15 minutes in a refrigerated centrifuge (Remi, India) at 4 0C. The aqueous phase was pipetted into fresh 1.5 ml eppendorf tubes, equal volume of chloroform: isoamyl alcohol mixture added and centrifuged similarly. The aqueous phase was again pipetted into a fresh 1.5 ml tube and 0.6 volume of chilled isopropanol was added. Content was gently mixed by inverting the tubes and kept in a freezer (4 0C) overnight for maximum precipitation of DNA. Tubes centrifuged at 13000 rpm for 15 minutes at 4 0C and the supernatant was decanted. The pellet was washed with 80% ethanol and centrifuged at 13000 rpm for 5 minutes. Extra alcohol was drained and the pellet was dried for half an hour in a vacuum until the alcohol smell disappears. Thirty microliter of TE buffer (10 mM TrisHCl, 1 mM EDTA; pH 8) was added and kept in room temperature overnight for dissolving of the DNA. DNA was stored in a deep freezer at – 20 0C until further use.

Quantitative PCR (qPCR) master mixture based on Taqman chemistry was purchased from the manufacturer Applied Biosystems Inc. USA. A pair of qPCR primers was designed based on the published partial sequences of 16SrRNA gene (EU635503, GQ121047) of WCLWD phytoplasma. The sequences of forward, reverse primers and the probe are **RTWF** 5' AGCCCCGGCAAACTATGTG, **RTWR** 5'AACGCTCGCCCCCTATG and 6' FAM-CAGCAGCCGCGGTA- MGB respectively. The PCR mixture consisted of Taqman master mix 7.5 µl, primers 0.15 µM each, water 0.57 µl, probe 0.15 µM and DNA 30 – 50 ng. Reaction mixture was dispensed into wells of optical PCR plates and covered with optical adhesive film prior to inserting into the instrument. The reaction was carried out in an Applied Biosystems 7500 Real-Time PCR instrument under standard conditions. Initially reaction mixture was optimized using 2 – 5 µl of DNA from white leaf disease affected sugarcane plants. Fifty one symptomatic coconut DNA, 2 white leaf sugarcane DNA and 4 healthy coconut DNA samples were used to get optimum DNA concentration in the mixture. The test was validated with 70 healthy and 132 symptomatic coconut palm DNA samples.

Results and Discussion

It was found that use of 2.0 µl of white leaf diseased sugar cane DNA sample (contained about 50 ng of DNA) produced pronouncing amplification plot. The cycle threshold values of different volumes of white leaf sugarcane DNA is indicated in Table 1. The results showed that increase in DNA content does not give significantly higher CT values.

	Sugarcane DNA 2µl	Sugarcane DNA 3µl	Sugarcane DNA 4µl	Injection water
CT value	18.6773	17.7254	17.6905	35.9934

Table 1. Average cycle threshold values of white leaf affected sugarcane DNA samples in Real-Time PCR

The best WCLWD affected coconut DNA volume was found to be 3.75 µl with lower cycle threshold value than other DNA volumes. In the validation reactions, initially false negative percentage was higher in number than positive samples which were collected during the dry season of the year. The percent positive symptomatic samples (n=51) collected during the dry period (February – September months) and rainy (wet) period (October –January months) of the year are given in Table 2. The four healthy samples didn't produce false positives.

Sampling Period	Symptomatic samples	Healthy samples
Dry	21.43(n=51)	0.0
Wet (rainy)	83.0 (n=151)	0.0

Table 2. Percent of symptomatic samples produced positive signals with qPCR tests during dry and wet periods

In the validation experiments, 96.2% of 132 DNA from symptomatic coconut palms were identified as affected and 98.1% DNA samples from healthy coconut palms identified as unaffected by qPCR. Only 3.8% affected and 1.9% healthy coconut samples were detected as false negatives and false positives, respectively. In biological tests, 95% accuracy is sufficient to consider as positive.

Perera *et al*., (2012) have reported that low detection rate of phytoplasma in symptomatic coconut palms was due to low titre of phytoplasma in the coconut tissues. It has been reported that phytoplasma translocate to different places of the affected plants during different seasons of the year, for example, grape vine phytoplasma move towards roots during winter season (Constable *et al*., 2003). Similarly, WCLWD phytoplasma also may move towards root-bole region where the temperature fluctuation is lesser than that of the canopy of the coconut palm. By staining of thin sections of rachilla, it was observed that the distribution of phytoplasma in all cells is not even (Fig. 1) (Wijesekara, and Fernando, 2013). It has also been observed that detection rates decreased in summer in Kerala, India with the Kerala Wilt affected coconut palms. The symptoms of Kerala Wilt disease in India are very much similar to that of WCLWD. The maximum detection rate of Kerala Wilt has been achieved during the winter months (personal communication with Dr. R. Manimekalai). Although phytoplasma enrichment procedure was tested for samples collected during the dry periods, the detection rate improvement was not significant. Wijesekara *et al*., (2013) has obtained less than 85% detection rate of WCLWD samples with the nested PCR.

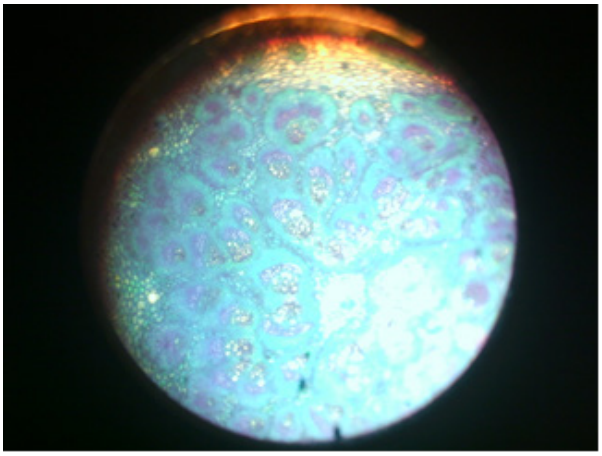

Figure 1. Thin sections of rachilla of WCLWD affected coconut inflorescence stained with Dienes stain showing uneven distribution of phytoplasma in sieve tubes (purple patches are phloem tissues with phytoplasma) (magnification 10x10)

It is essential to sequence PCR products generated by universal phytoplasma specific primers based on 16S rRNA gene, as many gram positive bacterial sequences tend to get amplified with those primers. The low rates of detection of symptomatic coconut samples in nested PCR are mainly due to lower sensitivity of nested PCR method. But current study

with RT PCR revealed that more than 96% of symptomatic palms detected as affected palms. This high rate of detection was achieved due to high sensitivity of the method and the specificity of the primers and probe developed for WCLWD. The undetected 4% symptomatic palms may be due to misidentification of nutrient deficient palms as affected palms or the phytoplasma titer in sampled tissue is very low. The detection rate of 98.1% in healthy palm category as unaffected is higher than that detect affected palms is a good indication of the accuracy of the test. Further, it indicates that qPCR primers RTWF/R together with 6' FAM MGB probe were more specific in detection of WCLWD phytoplasma (Table 3). Such a high detection rate is highly valuable in solving disputes in marking affected palms for eradicating the programme.

The DNA samples with higher titer of phytoplasma DNA show amplification with lesser number of PCR cycles (figure 2). The detection of sugarcane white leaf disease with same primers and the probe indicate that the regions of the gene used for the development of primers and probe are common to both phytoplasma species. These primers and probe can be considered as group specific primers and probes giving amplification with phytoplasmas with rice yellow dwarf group origin.

Sample type	Percent positive	False positive	False negative	Detection rate
Sympto-matic	96.2	0	3.8	96.2
Healthy	0	1.9	0	98.1

Table 3. Reaction of the samples in the qPCR validation experiment

Amplification of DNA samples above 35 cycles are considered as unspecified amplification or negative samples. According to the qPCR results, selection of WCLWD affected palms by visual observations by a trained person is of with high degree of accuracy.

Conclusion

WCLWD Phytoplasma detection rate varies with different seasons; the rainy and the dry season. Low detection rate was observed during dry period and detection improves with the onset of rains. The primers and the probe designed for qPCR are highly specific and it detects more than 95% of symptomatic palms as disease affected palms. Therefore, this test can be used for screening of suspected WCLWD palms in the disease eradication program. This study also confirmed that disease identification based on all three crown visual symptoms is also a quick and reliable alternative for the disease screening.

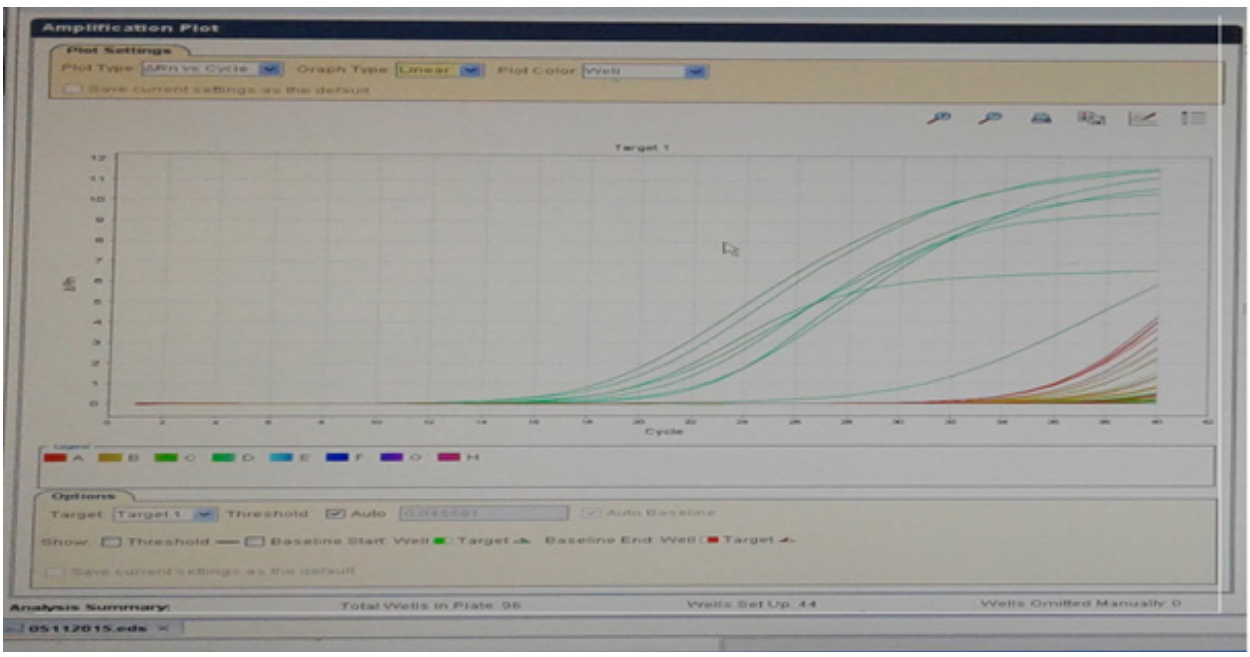

Figure 2. Amplification plot yielding by PCR reaction in Real-Time PCR machine

Acknowledgments

The authors gratefully acknowledge the financial assistance provided by FAO from the Technical Corporation Project 3303 to support WCLWD research and development program to carry out this experiment at CRI. The authors also wish to acknowledge the assistance from the Sugarcane Research Institute, Udawalawe by supplying white leaf sugarcane samples to this study whenever necessary.

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Cord 2020, **36**

The Coconut Industry: A Review of Price Forecasting Modelling in Major Coconut Producing Countries

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Abstract

The global supply and demand for coconuts and coconut-based products have increased tremendously over the past decades; hence, the industry has become one of the significant contributors to the economies of producer countries. However, similar to the other agricultural commodities, coconut has also been confronted by fluctuation in prices and thus accords importance for a reliable price modelling and forecasting techniques to ease the burden on the value chain actors. Therefore, the objective of this paper is to review the main approaches used in modelling and forecasting coconut prices, with an assessment of the strengths and weaknesses of each approach. The modelling techniques used in coconut price forecasting were mainly time series models dominated by univariate time series models. This type of models excessively confines the analysis to a single variable, despite the many interactions affected in a system of coconut pricing. The major drawback in existing price modelling studies is the absence of interacting factors such as prices, production, climatic variables, their interactions and the external factors as a system. Therefore, it is important to integrate the existing studies of coconut price modelling and forecasting with a system's approach by including other influencing variables to generate more realistic forecast values, allowing the industry to adopt its changing circumstances.

Key words: Coconut, modelling, price forecasting, time series models, system's approach

Introduction

Coconut (*Cocos nucifera* L.) is a versatile perennial tree crop with very important food value and other endless uses which pave the way for the emergence of a diversified set of industrial activities. Owing to the multifarious uses of different parts of the palm, it forms an integral component of the social, economic and cultural lives of nearly 80 million people in 92 countries (Naveena, *et al*., 2014). The economic importance of the coconut industry is manifold as well as a vital source of export earnings for the coconut producing countries. Coconut production is heavily confined to the Asia and Pacific region and the major producers in the world are Indonesia, Philippines, India, Sri Lanka and Brazil whereas the consumption is dispersed around the globe (Asian Pacific Coconut Community, 2016). During the past decade, the global demand for coconut had grown significantly (Figure 1) due to the increasing emphasis being placed on coconut water and Virgin Coconut Oil (VCO) due to their recently discovered health benefits (Rethinam, 2005). Moreover, strong niche markets are also emerging for coconut milk, coconut cream, coconut-based snacks, as well as coconut flour and coconut sugar, while the demand for traditional desiccated coconut and copra remains relatively stable (Sri Lanka Export Development Board, 2017). This driving up demand for the coconut-based products in the world market has created the derived demand for fresh coconuts.

Coconut needs price stability as a pre-requisite for its steady growth, because of its inability to short-run adjustments to price fluctuations unlike the seasonal and annual crops (Das, 1986). Being essentially a smallholder crop accounted for nearly 80 percent of the total holdings in major producer countries (Kalidas, *et al*., 2014; Pathiraja, *et al*., 2015), it further stresses the importance of having a stable price for the benefit of farmers' livelihood.

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Figure 1. World production and export quantities of fresh coconuts from 2000-2016 (FAO, 2017)

Rainfall and temperature are the major climatic factors influencing the coconut yield (Peiris, *et al*., 1995). Coconut production fluctuates due to immediate and lagged effects of adverse weather conditions resulting in instability of coconut prices. The consequences of such unusual price fluctuations are negative on the industry. For example, wide variations in prices erode the purchasing power of consumers, producers may also get wrong decisions and finally, the impact might make costly outcomes (Abeygunawardena, *et al*., 1996). Moreover, due to this characteristic uncertainty in the prices of coconuts and their products, the coconut industry has been trapped in a vicious cycle, a circle of low investment leading to low productivity and low return leading to low investment again and thus reinforcing themselves through a feedback loop.

Since economic conditions and consequently prices and price relations are ever changing, business judgments are based largely upon forecasts. The coconut industry itself also has come into a situation similar to the other agricultural commodities, where the prices are determined by both domestic and international market forces while leading to an increase in price variability, and accords importance to reliable price modelling and forecasting techniques (Jha and Sinha, 2013). Therefore, the objective of this paper is to review the main approaches used by the agricultural forecasters in modelling and forecasting coconut prices, with an assessment of the strengths and weaknesses of each approach. Some other relevant price models (of other crops) were also examined to see how the existing coconut price models were improved by approaches used in those models.

A Brief History

Agriculture Economics and Price Forecasting in a Nutshell

A scientific forecast is one made on the basis of a discovered systematic sequence of normal experience (Taylor, 1924). Forecasting is not a novel concept in agricultural economics, as it is one of the fundamental components of agricultural commodity trading. Consequently, price analysis and empirical modeling of price determination processes play a central role in research on agricultural markets. Therefore, forecasts of agricultural production and prices have been at the forefront of debate and controversy for over decades since the twentieth century (Allen, 1994; Li, *et al*., 2010) and it has been studied extensively since then.

There is a long history of econometric analysis starting with the partial analysis of a single commodity sector using single equation studies, which were then followed by multi-equation, multi-sectorial econometric studies. Expansion of the agricultural economic research towards forecasting production/ prices and estimating elasticity using econometric approaches were evident over the second quarter century from the existence of agricultural economics profession (Just, 1993). With the expansion of the complexity and flexibility of econometrics paradigm in the third quarter century, research focus had been moved towards policy, trade and environment and resource economics. To date, the dominant focus of agriculture economics research is on supply modelling, where most agriculture forecasting belongs.

The nature of the agricultural production and the historical relationship among the different groups of participants have made the agriculture different from most economic activities. Therefore, agricultural price modelling is different from modelling of non-farm goods and services (Allen, 1994). Production and prices of agricultural commodities are largely influenced by eventualities. Consequently, prices are unpredictable in case of natural calamities and hence, they are often random leadings to considerable risk and uncertainty in the process of agricultural price modelling and forecasting.

There are two commonly used basic approaches to forecasting in the literature. Those are structural models, which proceed from the first principle of consumer and producer theory, and time series models, where past observations of the same variable are analyzed to develop a model describing the underline relationships (Jha and Sinha, 2013). Agricultural application of modern time series methods did not appear till the 1970s; hence, price forecasts were largely made by conventional econometric methods in the earlier studies (Allen, 1994). However, during the past few decades, much effort has been devoted to the development and improvement of time series forecasting models (Jha and Sinha, 2013) and correspondingly, the methods have become increasingly complex. Meanwhile, comparing the forecasts from different methods were also progressed and quantitative accuracy with small errors, along with the turning point of forecasting power was considered for evaluating forecasting models.

Price modelling related to the coconut industry (fresh coconuts and coconut-based products)

Price forecasting of agricultural commodities has been extensively studied over decades and several different models have been developed (Li, *et al*., 2010). There is a considerable number of studies related to the coconut sector where similar modelling approaches have been applied. Table 1 summarizes the relevant literature on different approaches used for forecasting prices of fresh coconuts and coconut-based products.

The modelling technique that has been widely used in the literature to model coconut and other coconut products are time series models. The use of structural models describing how the future values of coconut prices are affected by specific economic drivers were hardly found in the literature. It appears that even though the structural models provide valuable insights into the determinants of commodity price movements, the contemporary picture is different in the reality. This may be due to the computational complexity and high data requirement of structural price forecasting models than time series models, which require less arduous data for consistent and up-to-date forecasting.

Abeygunawardena, *et al*., (1996) appeared to be the first and only group of researchers who forecasted one year ahead of retail and wholesale prices of coconut with a VAR model, a stochastic process model that captures the linear interdependencies among multiple time series, using twenty-year period monthly data (1973-1992). They have tested two models considering the explanatory power of the concerned variables. The first model is a two-variables VAR model defined by endogenous variables, retail and wholesale prices, where the second model, VAR-X, is an extension of the first adding lagged rainfall as an exogenous variable. According to their findings, the data was best fitted to the first model and one year ahead forecasts made using the model were statistically accepted. Moreover, they have concluded that though there is a lagged effect of rainfall on coconut yield, it has not contributed significantly on nut prices according to the model statistics. Even though, their results were in favor of two-variables VAR model, they have stressed on the importance of concentrating on developing a composite forecast model to make a better forecast for the future. More importantly,

Variable/s	Modelling approaches tested	Reference
Monthly retail and wholesale prices of coconuts in Sri Lanka	**BVAR** MVAR	Abeygunawardena, et al., (1996)
Monthly producer and retail prices of coconuts in Sri Lanka	GARCH **NARX**	Priyadarshani, et al., (2014)
Monthly coconut oil prices at Cochin market	TAR **TARCH**	Nampoothiri & Balakrishna,(2000) Nampoothiri, (2001)
National average prices of DC and CO, Retail and wholesale prices of fresh nuts	General Decomposition MA Double Exponential Smoothing **Single Exponential Smoothing** **ARIMA**	Rangoda, et al., (2006)
Monthly average prices of coconut, coconut oil and copra in three markets in India	Exponential smoothing methods **HWMS** **SARIMA** ANN	Indraji, (2014)
Annual prices of tea, rubber and coconut in Sri Lanka	ARIMA **VECM**	Nyantakyi, et al., (2015)
Monthly global prices of copra	TARMO	Ang, (2016)
Monthly average prices of coconut oil, copra and oil cake in Kochi	**HWMS** SARIMA	Elias, (2018)
Monthly wholesale price of coconut oil	**ARIMA**	Priyanga, et al., (2019)

Table 1. Summary of the coconut price modelling techniques in the literature
(The best model is shown with the bold letters)

* (BVAR – Bivariate Vector Autoregression, MVAR – Multiple Vector Autoregression, TAR - Threshold Auto Regressive, TARCH - Threshold Auto Regressive Conditional Heteroskedastic, MA– Moving Average, ARIMA – AutoRegressive Integrated Moving Average, HWMS - Holt-Winters Multiplicative Seasonal model, SARIMA – Seasonal AutoRegressive Integrated Moving Average, ANN – Artificial Neural Network, GARCH – Generalized Auto Regressive Conditional Heteroskedastic, NARX - Nonlinear Autoregressive Exogenous, VECM - Vector Error Correction Model, TARMO - Time Series Regression with ARIMA Noise, Missing Observations, and Outliers)

they have deflated both time series using food values of the Colombo Consumer Price Index and have used the prices in real terms for the analysis. Even though much of economics is about the consequences of changes in relative prices, sometimes theory and logic provide only general guides for empirical analyses that use relative prices. Therefore, the choice of deflators of commodity prices can change the time-series properties of the original series hence that could significantly impact empirical results (Deaton and Laroque, 1992; Harvey, 1993; Peterson and Tomek, 2000).

Another modelling attempt was made by Nampoothiri & Balakrishna (2000) to fit a TAR model to forecast monthly coconut oil prices using data from Cochin market, India from January 1978 to December 1996. They have concluded that the presence of non-linearity in the monthly oil prices series was well explained by a threshold model compared to a simple autoregressive (AR) model. However, later in 2001, the same authors (Nampoothiri, 2001) reported that wide and violent fluctuations existed on the same time series data were better handled by a composite model, which combined the TAR with an Auto Regressive Conditional Heteroskedasticity (ARCH) effect. This may be due to the presence of asymetric volatility in stock returns and a changing conditional variance of the time series. Notably, these two studies were focused on how a set of real data can be applied to different time series models

rather than modelling the coconut oil prices. This an example of showing the improvement of model accuracy by improving the dimensions of the modelling approach. TAR models perform better than AR because they handle non-linearity in the data as piece-wise linear models assuming that there are more than one linear regime in the observed data series. This has added new dimension to the simple linear AR process. Further improvement of TAR model structure with ARCH effect to represent the volatility jointly in a single schema enhances the applicability of the model.

Rangoda *et al*. (2006) have employed a range of conventional time series models including; general decomposition method, MA, HWMS, Single Exponential Smoothing method (SES), Double Exponential Smoothing method (DES) and ARIMA method to find the appropriate model to forecast prices of coconut and allied products. National average prices of desiccated coconut, coconut oil, and average retail and wholesale prices of fresh coconuts from January 1974 to December 2004 was used in the study. By considering the lowest MAPE, MAD and MSD, authors have concluded that ARIMA and single exponential smoothing method were better than other models to predict prices of fresh coconut, coconut oil and desiccated coconut. Of the methods tested, MA and SES techniques cannot be applied successfully when the data series show trends and seasonality but authors claimed that the magnitude of seasonal fluctuations and the positive trend of prices of the three products considered in the study were remarkably higher after the year 1983 compared to the preceding period.

Indraji (2014) attempted to model monthly prices of coconut oil and copra in Alappuzha, Kochi and Kozhikode markets in India from January 1990 to December 2015 and coconut prices at Alppuzha from January 1998 to December 2015. ARIMA, ANN and several different exponential smoothing models (SES, DES, Holt-Winters' additive (HWAS) and multiplicative) were employed on the study. Their analysis suggested that HWMS model was the best among the other fitted models in many occasions. Monthly prices of coconut oil, copra and coconut oil cake of Kochi market from January 1995 to December 2010 were reanalyzed by Elias (2018) using Holt-Winters Additive Exponential Smoothing and Seasonal ARIMA methods and claimed that Holt- Winter's method provided the best forecasting model. HWMS is among the most widely and successfully used smoothing methods for short term forecasting of financial time series. HWMS method is a complex expansion of the exponential smoothing method, since it exponentially smooths values for level, trend and seasonality adjustments. However, all the parameters in HWMS models are smoothing factors, which explain nothing much about the series behavior.

Even though Indraji, (2014) and Priyadarshani, *et al*., (2014) appears to be the first to use ANN approach for coconut price modelling, only Priyadarshani, *et al*., (2014) has got results in favor of ANN. Priyadarshani *et al*. (2014) used Generalized Auto Regressive Conditional Heteroskedasticity (GARCH) and Nonlinear Autoregressive neural network with exogenous inputs (NARX) models to forecast retail and producer price of fresh coconuts. The results showed that the NARX model was the most appropriate model to forecast both retail and producer prices of coconut during the study period. NARX neural network is a variant of a recurrent network, which can efficiently be used for modelling non-stationary and nonlinear time series. NARX models can better discover long time dependences than conventional recurrent neural networks but they still have limitations in learning long time dependences due to the "vanishing gradient".

Nyantakyi *et al*. (2015) examined the individual behavior of the prices of tea, rubber and coconut in Sri Lanka using ARIMA and the combined effects of these prices using Vector Error Correction models (VECM). They have shown that according to the VECM there may be a feedback relationship between all the three series and impulse response analyses revealed that there is a fairly strong correlation between them. Further, the authors concluded that ARIMA (0, 1, 3) was the best-fitted model for the annual price series of coconut from 1966 to 2009. Ang, (2016) explored the feasibility of Time series Regression with ARIMA noise, Missing observations and Outliers (TRAMO) model to forecast the global price of copra by using the monthly price of copra from January 2000 to June 2016. He concluded that the best-chosen model is ARIMA (3, 1, 0) (0, 0, 0)12.

The modelling techniques used in coconut price forecasting were mainly time series models including smoothing techniques such as single exponential, double exponential, HW's method, ARIMA model with or without seasonal component, VAR model and ANN approach. Smoothing

techniques and ARIMA models were comparable and both methods were successful in forecasting coconut prices (Rangoda, *et al*., 2006). On some occasions, HW smoothing models outperform ARIMA (Elias, 2018) because they can quickly adapt to minor changes in the market condition. However, exponential smoothing methods are essentially methods of fitting a suitable curve to historical data of a given time series and hence they are now supplemented by the other advanced economic forecasting methods (Gujarati and Porter, 2008). Generally, in econometric forecasting, ARIMA is well accepted as a better technique than other considered methods (Meade, 2000; Nochai and Titida Nochai, 2006; Harris, *et al*., 2012; Jha and Sinha, 2013; Adebiyi, *et al*., 2014), which is also supported by Rangoda, *et al*. (2006), Ang, (2016) and Priyanga, *et al*., (2019) proving that ARIMA is the better method in forecasting coconut prices. However, Ang, (2016) has recommended a multivariate analysis to enhance the reliability of the forecasting because ARIMA is only defined by the present and lagged prices as explanatory variables. According to the literature, prices of coconuts and other coconut-based products are induced by the factors such as cross-price elasticity of coconut oil with respect to other edible oil prices (substitution effect), global market prices and the supply-demand relationship (Das, (1986), Estal, (2014), Jayalath and Weerahewa, (2014)). Therefore, the incorporation of such relationships into the modelling approach has a potential for improvement of applicability and better forecasts.

There are several studies that have used the above discussed time series forecasting techniques in other plantation crops such as tea and rubber. These studies also have provided successful models and prediction outcomes favoring the use of time series methods (Aponsu and Jayasundara, 2012; Hettiarachchi and Banneheka, 2013; Ikonya, *et al*., 2014; Cherdchoongam & Rungreunganum, 2016; Induruwage, *et al*., 2016; Liu and Shao, 2016; Hussain and Ali, 2017). After reviewing several studies on tea price modelling, Gunathilaka and Tularam, (2016) have concluded that VAR model appears to be the more appropriate method for modelling tea prices as it allows to incooperate a group of interacting time series variables to explain the dynamic relationships among these time series in the system. Furthermore, this framework permits to include other variables such as climatic variables to quantify the likely impacts. However, as the real-world prices of agricultural products and their underlying market fluctuations are often non-linear in nature, much attention has focused on ANN in recent years as an alternative technique for forecasting in economics (Li, *et al*., 2010; Jha and Sinha, 2013). Several studies that have compared the ANN with other time series approaches like ARIMA have proven that ANN performs much better than the other models (Hettiarachchi and Banneheka, 2013; Jha and Sinha, 2013; Hussain and Ali, 2017). ANN allows to modelling complex non-linear relationships between the prices and its determinants such as meterological and seasonal variables, food safety, risk and incertainity etc. (Li, *et al*., 2010).

Due to time series approaches used, none of the studies above could explain the real reasons/causes for getting a particular forecast, even though the models were able to predict the future with minimum error based on the past observations. However, a vulnerable system like the coconut industry needs to be adjusted for potential uncertainties based on the most influential factors deciding the future prices in different situations. Therefore, the price changes in coconut-based products should be studied together as interdependent sub-systems and modelled based on a systems' approach for a better understanding of the system dynamics. Selection of suitable forecasting methods is very crucial to handle increasing variety and complexity to generate reliable and accurate forecasts. As each method has its limitations depending on the situation, the selection of the forecasting technique should be carefully done to get the maximum accuracy.

Given the complexity of the coconut industry, several scholars have attempted to develop an economic framework to model the industry. De Silva (1985) hypothesized the Sri Lankan coconut industry by adopting a Partial Equilibrium Model (PEM); a condition of economic equilibrium that has taken into consideration only the considered segment of the market to attain an equilibrium, while all other segments of the market were kept constant. However, this study was limited to graphical illustrations as the inadequacy of data prevented estimation of basic coefficients required for the analysis. Considering three major products in the coconut market; culinary nuts, coconut oil and desiccated coconut, Samarajeewa (2002a, 2002b) also estimated a PEM for the coconut market in which the supply and demand relationships in each market were linked using the equilibrium price and the system of equations were econometrically estimated. More recently,

Pathiraja *et al*.,(2017) developed and tested an Equilibrium Displacement Model, a type of PEM, for the coconut market. Abeysekara *et al*.,(2020) re-estimated the PEM adopted by Samarajeewa *et al*., (2002) to analyze the changing directions of the supply-demand relationships in the coconut market. However, the primary objective of all these studies was to either analyze the economic results of policies affecting the coconut industry or to analyze the economic impact of different climate scenarios. More importantly, PEM models are static and hence do not serve the purpose of price forecasting even though they capture all the equilibrium relationships in the specified market. These can however be used to estimate the economic impact of exogeneous shocks (i.e.policy, climate scenarios, etc.). The PEM equates supply and demand in one or more markets so that prices stabilize at their equilibrium level. Therefore, once calibrated, these can be used to simulate the point forecast of equilibrium price.

Conclusions

Price movement and volatility forecasting in coconut-based products have been extensively studied using univariate time series techniques. The use of bivariate and multivariate techniques in this domain is minimal. Univariate time series models are easy to handle as they forecast the future by evaluating the past observations (trends and seasonality) of the dependent variable in the model. As they do not consider the interactions between variables, use of these types of models excessively confines the analysis to a single variable, despite many interactions affected in a system of coconut pricing. Univariate models, therefore, are less explicable about the observed changes regardless of the accuracy of the forecast. Nevertheless, various exogenous variables that influence the endogenous variables of the coconut pricing system are evidenced in the experimental literature. Among them, anomalies in weather parameters, substitution factors, import factors, and different government policies may significantly contribute to the system of price determination. Analyzing the consistency of such observational data may also be useful in determining the theoretical relationship between the variables to be used in the modelling. Therefore, the incorporation of influential exogenous factors into the model may yield a better understanding of the situation and uncertainties.

Among the multivariate techniques tested on coconut pricing, VAR and ANN appeared to be the most successful methods. The VAR technique, one of the most successful and flexible models for analyzing multivariate time-series, has the ability to model the non-structural relationship of coconut price along with other time series variables. ANN models that can model the complex nonlinear relationships between price and its determining factors have been frequently applied in modelling prices of other agricultural commodities and they have provided successful models and prediction outcomes. Several attempts have been made to conceptualize an economic framework to model the coconut industry using PEM, thus provide only a point forecast. However, it is noted that prices, production, climatic variables and other factors as a system have not yet been considered in the coconut price modelling and forecasting and hence appears to be a major gap in the existing studies. Therefore, it is important to extend the existing studies of coconut price modelling and forecasting to include the other interacting variables to generate more explicable forecast values allowing the industry to adapt its uncertain circumstances.

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Cord 2020, **36**

Design and Development of Semi-Direct Copra Dryer for Flat Terrain

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Abstract

The study was conducted to design and develop a semi-direct dryer for flat terrain. The components of the dryer were the drying bed, plenum chamber, tunnel and firing chamber. It has a capacity of 2,000 nuts and the husks from the nuts were used as fuel for the dryer. The means of the average drying temperature on the front, middle and rear portions of the drying platform were 56.5°C, 58.2°C, and 58.4°C, respectively. The average time of drying in bringing down the moisture content of the copra from 50% to 12% wet basis was 24 hours using 66.30% of the husks. As of October, the total cost of the dryer with shed was P 62,000.00 and the computed break-even cost was P 1.82/kg. This dryer provides coconut farmers an alternative to existing dryers particularly the semi-direct since it is only suitable for rolling terrain.

Key words: Design, development, semi-direct copra dryer, terrain, copra

Introduction

Importance of the Study

The "tapahan" dryer is commonly used by the coconut farmers in the Philippines in copra processing. According to Raghavan (2010), the basic features which make the "tapahan" dryer preferred by farmers are: high thermal efficiency of the dryer, low cost of construction, simplicity of the design and low cost of fuel. However, de Castro (1978) stated that copra made from "tapahan" are most often unevenly dried and usually blackened by soot. Sudaria (1993) also mentioned there is a high probability of the "tapahan" together with the copra getting burned because the firing place is directly under the drying bed.

Sudaria (1993) developed a semi-direct type copra dryer. It has the same heating principle as the "tapahan" dryer but the firing chamber is away from the drying platform connected by a tunnel. It was also cheap using only materials available on the farm such as coconut lumber, coconut fronds and bamboo. However, this dryer is only suitable when constructed on rolling terrain. Problems occur in the construction if the terrain is flat and the water table level is high. The firing place reaches the water table and the slope of the tunnel connecting the firing place to the plenum chamber is quite difficult to excavate resulting in an uneven heat distribution on the drying bed. There are developed copra dryers suitable for flat terrain particularly indirect dryers. The problems of this dryer were low thermal efficiency and very expensive (Escalante, *et al*. 1977). This dryer was designed to provide farmers an alternative to traditional and existing dryers particularly the semi-direct dryer.

Objectives of the Study

The general objective of the study was to design and develop a semi-direct dryer, evaluate the performance and determine the break-even cost of the dryer for flat terrain.

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Materials and Methods

Design Consideration

The following criteria were formulated based on the information collected from the coconut farmers, existing copra dryers, and personal experience to develop an up to standard copra dryer: a. cost should not be higher than any alternative dryers for flat terrain, b. construction should not require special tools, c. easy to use and maintain, the lifespan of the essential dryer components should be at least five years, a dryer can accommodate 2,000 coconuts per batch, heat distribution on the drying platform must be comparatively equal, thermal efficiency of not less than 12%, fuel usage is lower than 90% of the husks of nuts loaded and the performance is comparable to the existing semi-direct dryer.

Design, Construction and Testing

The semi-direct dryer for rolling terrain of Sudaria (1993) was the basis of the design. Modifications were made for it to be suitable for flat terrain. The design of the dryer was prepared using AutoCAD 2016. The dryer was constructed at Visayas State University, Visca, Baybay City, Leyte. The materials used in constructing the dryer were bamboo slats, hollow blocks, cement, sand, gravel, deformed round bars, and lumber. The components of the dryer were the drying bed, plenum chamber, tunnel and firing chamber.

Drying Bed: It had a dimension of 320 cm x 240 cm x 70 cm. Bamboo slats were used in the construction of the drying platform and laid 2 to 3 cm between slats. Beneath the bamboo slats were nine 5.08 cm x 15.24 cm lumber. The drying bed was elevated 215 cm above the ground and stairs were provided on the perimeter of the drying platform. The sides of the drying bed were made from 10.16 cm x 20.32 cm concrete hollow blocks supported by 10 mm reinforcing deformed round bars. The drying bed has a pathway of 75 cm x 75 cm near the center.

Plenum Chamber: It was made of compacted soil and has a spoon-like design with a parabolic curve with 1 m center depth from the drying platform.

Tunnel: It had an inner dimension of 55 cm x 55 cm with 10% inclination. It was made from 10.16 cm x 20.32 cm hollow a blocks reinforced with 10 mm deformed round bars. The tunnel has a wide opening with dimension of 150 cm x 240 cm near the drying platform. The tunnel has a total length of 300 cm.

Firing Chamber: The firing chamber was connected to the tunnel. It has a dimension of 80 cm x 190 cm.

A preliminary test was done after the construction. The dryer was tested without load using dry coconut husk as fuel. Laboratory thermometers were placed on the front, middle and rear portions of the drying platform. The temperature was monitored for 2 hours. The dryer was further modified and tested until the temperature on the different portions of the drying platform was comparatively even and ready for final evaluation. Figure 1 shows the orthographic views of the dryer.

Preparation of Coconuts

Mature nuts of Baybay Tall variety were used in this study. The coconuts were de-husked using a traditional dehusker. The removed husks were kept in a dry place and used as fuel for the dryer. The shell was cracked open by using a machete resulting in two almost equal "cups". The coconut water was drained off and the "cups" were dried with the meat still attached to the shell. The "cups" were immediately loaded on the drying platform with the first layer in a vertical position. The succeeding layers were placed in an inclining position and the topmost layer was placed with the kernel facing down.

Performance Evaluation

Twelve samples were collected randomly from the pile. Each sample was weighed using a digital weighing scale to determine the initial weight of the meat with the shell. The samples were labeled and placed on the bottom and top portion of the pile on the different location (right and left side of front, middle and back portion) of the drying platform. Laboratory thermometers were placed near the samples to determine the drying temperature and outside the dryer to determine the ambient temperature.

During the drying process, the weight of the samples and temperature were measured and recorded at an hourly interval. A constant feeding rate of 9 to 11 husks per 10 minutes of drying was observed to control and regulate the drying temperature. The drying was accomplished after 3 stages, i.e. 8 to 9 hours

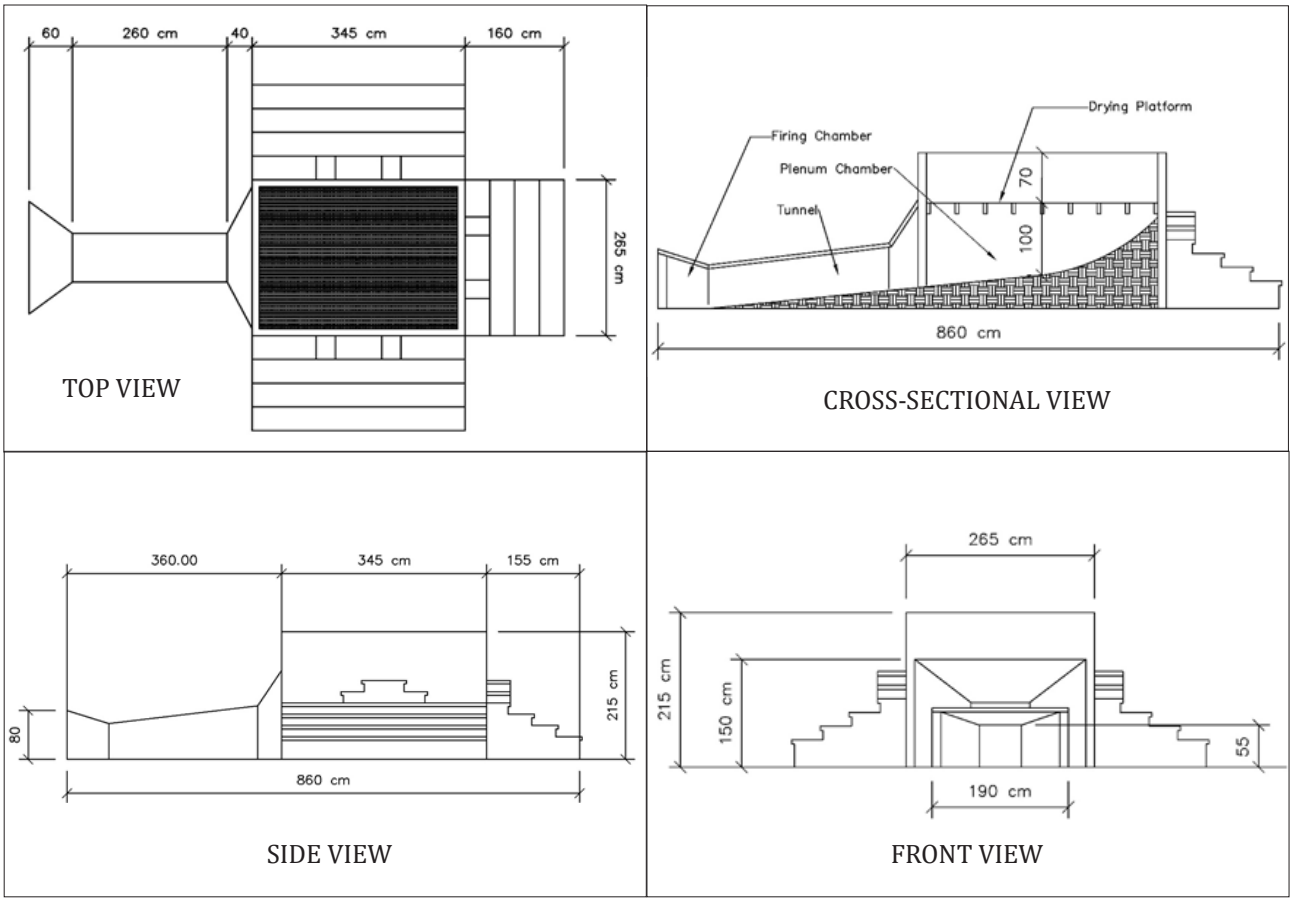

Figure 1. Orthographic view of the dryer

of continuous firing at daytime and allowed to cool-off during the night. At the 3rd stage of drying, the shell and meat were separated using a scooper when the shell and meat were partially detached. The weight of the shell was measured. The drying was stopped when the copra had reached a moisture content of 12%, the moisture content level where local coconut farmers sold their copra to village traders at a discounted price. This is referred to as the "pasa" system.

The drying was replicated thrice using 2,000 nuts per replication. The total drying time was recorded. The used coconut husks were counted to determine fuel consumption. The appearance of the copra produced was observed. The temperature difference on the different portion of the dryer was compared using one-way analysis of variance.

Estimation of Drying Efficiency

The drying efficiency of the semi-direct dryer was estimated using equation 1.

Eqn. 1

$$Thermal\ Efficiency\ (\eta_t) = \frac{\varphi\lambda\ (M_o - M_f)}{WC\ (100 - M_o)} x\ 100$$

Where, Mo = initial moisture content of coconut (%, wet basis), Mf = final moisture content (%, wet basis), φ = quantity of the final dried product at Mf moisture content (kg), λ = latent heat of vaporization of water in kcal/kg., W= quantity of fuel used (kg), and C = calorific value of fuel used (kcal/kg) (Sing *et al*., 1999).

Determination of Break-even Cost

In determining the break-even-cost (BEC), the procedure discussed by Henderson *et al*. (1976) was used. It considered both the cost of the dryer and its output. BEC was computed in terms of amount per kilogram of copra.

RESULTS AND DISCUSSION

Semi-direct Dryer for Flat Terrain

The components of the semi-direct dryer were the drying bed, plenum chamber, tunnel and firing chamber. The drying bed had a volume of 320 cm x 240 cm x 70 cm with a capacity of 2,000 nuts. A passageway was made on the drying bed to ease the maintenance of the dryer. The firing chamber was 300 cm away from the drying platform connected by a tunnel. The tunnel connects the plenum chamber and the firing chamber and facilitates the flow of the hot air. The size of the tunnel was 55 cm x 55 cm for the first 260 cm and the remaining 40 cm was an enlarged cross-section going to the drying platform which regulates the airflow to have an equal distribution of heat throughout the drying bed. It had an inclination of 10% which ensures that the burned fuel from the firing chamber is not blown into the plenum chamber where it could burn the copra. The plenum chamber had a spoon-like design with a 100 cm center depth from the drying platform. The spoon-like design of the plenum chamber allows the dryer to evenly distribute the hot air including the corners of the drying platform which results to an equally cooked copra. A shed was constructed for continuous drying throughout the rainy season. Figure 2 shows the semi-direct dryer for flat terrain.

Development of the Semi-direct Dryer

The semi-direct dryer of Sudaria (1993) was modified particularly the inclination of the tunnel. Sudaria (1993) mentioned that the tunnel inclination for semi-direct dryer would be around 20 percent. However, the dimension was not suitable for the flat terrain. The height of the drying bed is 260 cm which is difficult when loading and unloading the copra. The tunnel inclination was modified to 10 percent to achieve a feasible height of 215 cm of the drying bed. Other dimensions i.e. volume of the drying bed, tunnel size, and firing chamber size were followed.

A preliminary test revealed that the heat distribution on the drying platform was uneven. The temperature on the front section of the drying platform was very high compared to the temperature on the middle and back sections of the drying platform. The temperature difference between the front and back section of the drying bed is about 15°C to 20°C. According to Sudaria (1993), the size and inclination of the tunnel affect the heat distribution on the drying platform. The total length of the tunnel was 300 cm. It was divided into two sections. From the firing chamber, for the length of 260 cm the cross section of the tunnel was 75 cm x 75 cm. The remaining 80 cm was an enlarged cross section going to the drying platform. By decreasing the

Figure 2. Semi-direct dryer for flat terrain

size of the tunnel, the air velocity increases thus the hot air could reach the back section of the dryer. The size of the tunnel was then reduced to 55 cm x 55 cm with a length of 260 cm and the remaining 40 cm was the enlarged cross section as shown in Figure 1. Reducing the tunnel size further would cause difficulty in maintaining the tunnel. The dryer was tested again with the modification. Result of the test revealed that the heat distribution on the different sections of the drying platform was comparatively equal with a temperature difference of 2°C to 3°C.

Performance of the Semi-direct Dryer

The average drying temperatures on the drying platform were 56.5°C, 58.2°C, and 58.4°C, on the front, middle and back portion, respectively. Figure 3 illustrates the mean temperature generated during drying. Table 1 shows the average temperature on the different portions of the dryer and the ambient temperature. Result of the one-way analysis of variance revealed that there are no significant differences of the temperature on the different portion of the dryer. The temperature at the front, middle, and back portions of the drying platform were comparatively equal. The average drying time to bring down the moisture content from 50% to 12% was 24 hours using 66.30% of the 2,000 coconut husks. Figure 4 shows the drying curve of the copra. Sudaria (1993) reported that the existing semi-direct dryer has an average drying time of 23 hours to bring down the moisture content from 50% to 12% using 62.15% of the 2,000 coconut husks.

	Replication			Mean
	1	2	3	
Front	55.70	56.60	57.20	56.50 a
Middle	57.90	57.70	59.00	58.20 a
Rear	58.20	57.60	59.40	58.40 a

*Means having the same letters are not significantly different from each other at 5% level

Table 1. Average temperature (°C) of the different portions on the drying platform

Drying Efficiency of the Semi-direct Dryer

The estimated thermal efficiency of the semi-direct dryer calculated using equation 1 was 12.6% to 14.3%. The existing dryers specifically the "tapahan" and modified kukum dryer have a thermal efficiency of 12.7% while the COCOPUGON dryer has a thermal efficiency of 15% (Dippon and Villaruel, 1996).

Appearance of the Copra

The color of the copra ranged from light to dark brown as shown in Figure 5. The appearance of the copra was similar to the copra produced by the existing semi-direct dryer.

Break-even Cost Analysis

The factors considered in getting the BEC were the cost of the structure, labor and the output of the dryer. The total cost of the structure (including the shed) and labor as of October 2020 was P 62,000.00. The total weight of the copra produced from 2,000 nuts was 504 kg. The computed break-even cost of the dryer was P 1.82/kg copra. The break-even cost of the existing semi-direct dryer and "tapahan" dryer ranges from P 0.60/kg to P 1.00/kg. Though the break-even cost

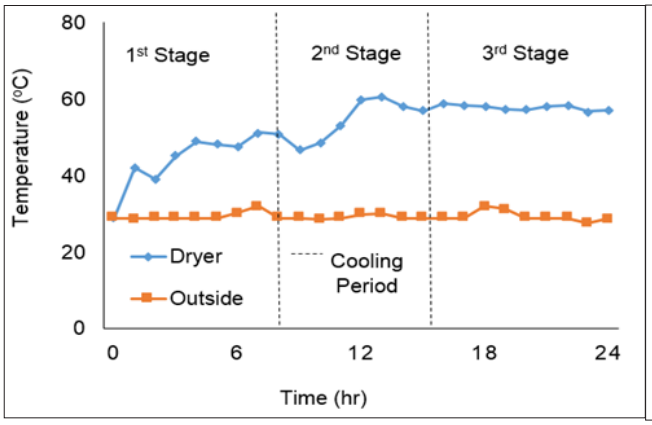

Figure 3. Drying temperature of the dryer

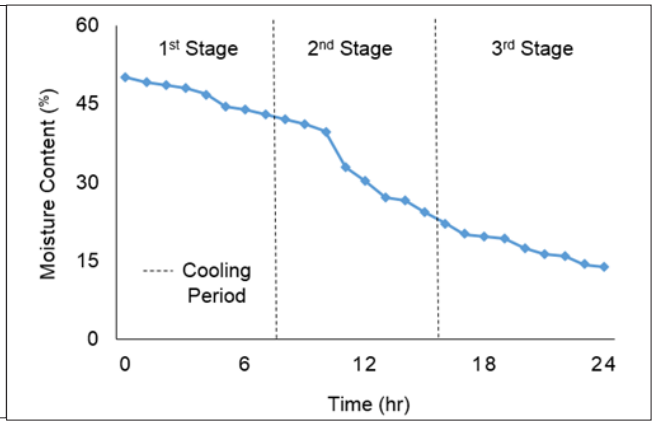

Figure 4. Drying curve

Figure 5. Appearance of the copra

of the existing dryer is lower than the new drier, when considering the life span of new drier, it has added advantage for the processors.

Summary, Conclusion and Recommendation

The study was conducted to design and develop a semi-direct dryer for flat terrain. Specifically, it aimed to design and construct a semi-direct dryer for flat terrain, to evaluate the performance of the dryer and to determine the breakeven cost of the dryer.

The components of the dryer were the drying bed, plenum chamber, tunnel and firing chamber. It had a capacity of 2,000 coconuts. The fuel used was the husk from the dried coconuts. The means of the average drying temperature on the front, middle and rear portions of the drying platform were 56.5°C, 58.2°C, and 58.4°C, respectively. The average time required to bring down the meat moisture content from 50% to 12% was 24 hours using 66.30% of the coconut husks. The estimated drying efficiency was 12.6% to 14.3%. The total cost of the materials was P 62,000.00 with a computed break-even cost of P 1.82/kg of copra. The color of the copra ranged from light to dark brown. The performance of the dryer was comparable to the existing dryers. It is recommended as an alternative to traditional and existing dryers, particularly the semi-direct and indirect dryers.

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Evaluation of Nutritional Composition of Defatted Coconut Flour Incorporated Biscuits

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Abstract

Defatted coconut kernel is the major by-product of the dry method of virgin coconut oil (VCO) processing which includes high fiber content. It is ground into a fine powder, has a high potential to use as a composite matrix for baked food items with wheat flour. The aim of this research is to compare nutritional characteristics of defatted coconut flour incorporated biscuits (CF), desiccated coconut incorporated commercial biscuits (DC) and wheat flour-based commercial biscuits (WF) using white bread (WB) as a reference. Proximate composition (moisture, ash, crude fat, crude protein, crude fiber and carbohydrate), Hydrolysis Index (HI) and Predicted Glycemic Index (PGI) of samples were performed through standard in-vitro analysis methods. Results of the proximate analysis revealed that moisture (36.67±0.16 %) and protein (13.35±1.17 %) content of bread were significantly ($p<0.05$) higher while fat, fiber and ash content of CF incorporated biscuits were significantly ($p<0.05$) higher with the values of 26.67±1.87 %, 3.53±0.10 % and 4.70±2.61 % respectively. Free sugar glucose content (FSG) of WF was observed the highest significant ($p<0.05$) value of 5.88±1.03 % while the highest amount of rapidly available glucose (RAG) (81.45±5.27 %), slowly available glucose (SAG) (59.81±7.58 %), total glucose (TG) (99.16±5.56 %) were observed in reference food of bread. The PGI of three biscuit types belonging to the medium glycemic food with the values of 60.84, 64.53 and 62.90 respectively for CF, DC and WF treatments.

Key words: Biscuits, defatted coconut flour, glycemic index, in-vitro digestion

Introduction

Coconut (*Cocos nucifera*) is a widely used raw material in food preparation especially in tropical countries. It is rich in nutrients such as fiber, saturated fat, protein, carbohydrates and minerals such as sodium, potassium, calcium and magnesium. Virgin coconut oil is one of the most valuable products which is extracted from fresh coconut meat without brown testa (paring) with low temperature (dry extraction cold-press process) or without the use of heat (wet extraction process) (Thaiphanit and Anprung, 2016). Consumption of medium-chain fatty acid (MCFA) such as VCO reduces the risk factor of cardiovascular diseases (Oh K *et al.*, 2005), lowers the blood pressure (Nurul-Iman *et al.*, 2013) and blood sugar (Babayan, 1987). The MCFA presents in the VCO can be acted as an energy source for the brain through metabolizing it into the ketone bodies which are converted to acetyl Co-A to produce ATP and it acts as precursors for acetylcholine in neurons (John *et al.*, 2020). Moreover, pure coconut scent is preserved in VCO with a high level of antioxidants and vitamins to improve the anti-carcinogenic activity of the human body (Nevin and Rajamohan, 2004). During virgin coconut oil production defatted edible solid by-product is removed and which is called defatted coconut residue or oil cake ("poonac, Punnakku") can be milled into flour. The coconut flour can provide a nutritious and healthy source of dietary fiber. Trinidad *et al* (2003) reported that coconut flour plays a role in controlling cholesterol and sugar levels in the blood and in prevention of colon cancer.

Consumption of food with high fiber is very essential for safeguarding from prevalent non-communicable diseases such as elevated cholesterol, diabetic and cancer. Therefore, people more concern about the ingredients in their diet especially low sugar and high fiber. However, fast-moving lifestyle accelerates fast food consumption such as biscuits, cake and other bakery products. The demands of these types of products are increased because of less perishability, durability, and easy handling than high perishable foods.

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Most of the fast foods are made of wheat (*Triticum aestivum L.*) flour. Wheat flour contains 78.10 % carbohydrates, 2.10 % fat, 2.10 % minerals, 14.7 % protein. The quality of wheat flour-based food is high due to the presence of wheat protein gluten (Okpala and Egwu, 2015). Ingredients that are added to the biscuit and processing method can alter the nutritional composition of the final biscuit. Defatted coconut flour is a fiber-rich ingredient and it can alter the nutritional composition of the product compared to the traditional wheat flour-based product. Therefore, Glycemic Index food which is an important characteristic can be altered by the substitution of wheat flour with defatted coconut flour.

Glycemic Index (GI) is defined as the incremental area under the β-glucose response curve (IAUC) of a tested meal containing 50 g of digestible carbohydrates and the incremental area under the β-glucose response curve of the standard food (50 g pure glucose) (IAUCS) (Rudolf *et al.*, 2004). Diet with high GI causes elevated blood glucose level and such food has been associated with the risk of chronic heart diseases. Therefore, the identification of the glycemic index of a food is very crucial for diet management. Generally, the GI of food is determined by in-vivo clinical studies even though it is a time and money-consuming method. However, to predict the GI of a food product through in-vitro digestion that occurs in the upper gastrointestinal tract of humans is used as a promising alternative for clinical GI measurement (Woolnough *et al.*, 2008). The aim of this research is to determine nutritional composition and prediction of the glycemic index (PGI) of defatted coconut flour incorporated biscuits the same as to desiccated coconut incorporated commercial biscuits and wheat flour-based commercial biscuits with reference to white bread through in-vitro analysis.

Materials and Methods

Materials

Defatted coconut flour added biscuits (CF) were prepared in Coconut Processing Research Division at Coconut Research Institute, Sri Lanka. The desiccated coconut added biscuit (DC), wheat flour added biscuit (WF) and white bread (BR) as a reference were purchased from the Cargill Food City supermarket at Dankotuwa, Sri Lanka. The enzyme of pepsin, amyloglucosidase, invertase and pancreatin were purchased from sigma. The poly propylene tubes (50 ml), Fan forced Oven (JEIO TECH (OF – 22G), Analytical balance (OHAUS), Randall hot extraction apparatus (Behr E 6), FIWE extraction unit, Shaking water bath (SWBR17-2), Spectrophotometer (UV-1800 Shimadzu) were used.

Methods

Preparation of defatted coconut flour-based biscuit

A dough of biscuits was prepared by mixing 300 g defatted coconut flour and 700 g wheat flour as the main matrix for biscuits development. Other ingredients: baking powder (20 g), margarine (600 g), sugar (500 g), egg (50 g) and salt (5 g) was measured and mixed well in a bowl in a machine of dough mixture (model- Sherry 8p-800) in 20 min. Then the dough was leveled by a roller and was cut using a mold of circular shape (diameter 2 cm) and placed on an oil paper in a stainless-steel tray. The biscuits were baked for 15 minutes at 160 oC in a pre-heated oven and were allowed for cooling and were packed in triple laminated aluminum pouches.

Proximate composition

The proximate composition (moisture, ash, crude fat, crude protein, crude fiber and carbohydrate) of four samples was determined (AOAC, 2005) with four replicates.

In-vitro measurement of different sugar fractions

Free sugar glucose (FSG) contents of each biscuit were determined by Nani *et al.* (2017) method. Biscuit samples with 0.5 g carbohydrate were placed in a polypropylene tubes and 5 ml of 0.5 M sodium acetate buffer (pH 5.2) were added. The contents were shaken well. The samples were incubated at 100 oC in a boiling water bath for 30 minutes and cooled to room temperature. The glucose contents of the samples were measured by using a glucose determination kit (GOD PAP, France). GI of three biscuit samples with reference to white bread were determined according to Englyst's protocol with modifications (Englyst *et al.*, 1995). Minced biscuit with 0.5 g carbohydrate was placed in polypropylene tubes and were subjected to a mixture of enzymatic digestion with mechanical disruption by small glass balls. Then, samples were incubated at 37 °C in a shaking water bath to continue the hydrolysis and 1 ml of samples

Treatments	Moisture (%)	Protein (%)	Crude Fat (%)	Ash (%)	Crude Fiber (%)	Carbohydrate (%)
BR	36.67±0.16a	13.35±1.17a	2.60±0.11d	2.43±0.12b	0.28±0.09c	44.67±1.34d
CF	3.04±0.08b	3.67±0.21c	25.67±1.87a	3.53±0.10a	4.70±2.61a	59.40±3.75c
DC	0.89±0.08d	4.76±0.42b	18.21±0.15b	1.65±0.20d	3.08±0.22ab	71.42±0.86b
WF	2.28±0.11c	4.71±0.38b	11.24±1.44c	2.04±0.20c	1.46±0.57bc	78.28±0.92a

Table 1. Proximate composition of resulted biscuits, commercial biscuits and white bread

Values are Means ± standard deviations and different superscripts in the same column are significantly different ($p<0.05$), BR: White bread, CF: Defatted coconut flour-based biscuits, DC: Desiccated coconut added biscuits, WF: Wheat flour-based biscuits.

were taken from each treatment at certain time intervals (0, 20, 30, 60, 90 and 120 minutes) to determine the glucose content. The glucose content of sample after 20 min was measured as G20 – Rapidly Available Glucose (RAG) and glucose content of the sample after 120 min of digestion was designated as G120 – Slowly Available Glucose (SAG). After 120 min, the remaining samples were treated with additional enzymes and the temperature was increased to 100 °C to complete the hydrolysis followed by centrifuging at 1500 xg for 2 min to obtain a clear supernatant and thus, Total Glucose Content (TGC) of the samples were determined. To measure the glucose content at each time for specific digestion, an aliquot of (200 µl) of the sample was mixed with 4 ml of absolute ethanol to stop the hydrolysis. Glucose content of the sample was determined using a glucose determination kit (GOD PAP, France) based on the procedure given by the GOD, PAP, France and the absorbance was measured at 505 nm using a spectrophotometer (UV-1800 Shimadzu).

Calculations and statistics

RAG, SAG and various starch fractions were calculated by using the following equations.

1-Rapidly Available Glucose (RAG) = G20

2-Slowly Available Glucose (SAG) = G120 – G20

3- Free Sugar Glucose (FSG) = G30

The results were converted to starch by multiplying the percentage glucose concentration by a factor (0.9). Then percentages of starch hydrolysis were built for each sample and reference food over the incubation time. The area below the hydrolysis curve (AHC) was estimated by integrating the hydrolysis curves. The Hydrolysis Index (HI) was calculated as the ratio between the AHC of each sample and the AHC of glucose and expressed as a percentage. The GI of the samples were estimated according to the equation of GI = 39.71 + 0.549 HI (Goni *et al.*, 1997). Statistical analysis (ANOVA) was performed using the MINI TAB system (Version 16.0). Tukey's tests were used for comparison of means at $p<0.05$.

Results and Discussion

The results of proximate composition show significant differences for moisture, protein, crude fat, ash, crude fibre and carbohydrates contents of biscuits (Table 1).

Moisture

The moisture content of food indicates the water activity which is responsible for the shelf-life stability of processed food. Adding more water into the dough of bread increases the water content of bread to increase the bread yield and profit (Miller *et al.*, 2008). That's why bread has significantly ($p<0.05$) higher moisture content (36.67±0.16 %) compared to the three types of biscuits (Table 1). Results showed that CF has significantly ($p<0.05$) higher moisture content (3.04±0.08 %) than DC (0.89±0.08 %) and WF (2.28±0.11 %) biscuits samples. The reason may be due to the high water absorption ability of coconut flour.

Protein

Proteins are essential macromolecules for the development of biological organs. Amino acids are monomers of the protein molecules. The BR has significantly higher protein content (13.35±1.17 %) (Table 1). The protein content of DC (4.76±0.42 %) and WF (4.71±0.38 %) is

significantly ($p<0.05$) the same while CF shows a significantly low protein content (3.67±0.21 %). Thirty percent substitution of wheat flour from defatted coconut flour did not affect the increment of protein percentage in defatted coconut flour added biscuits even defatted flour has a higher protein content of 21.76 % (Yalegama *et al.*, 2013).

Fat

The fat content of food provides energy and essential fatty acids to boost the body function. Source of fat (Animal or plant origin) determine the health aspects of human while creating non-communicable diseases such as elevated cholesterol and heart diseases (Zheng F. M. and Yeong Y.L, 2016). Coconut flour contains significantly high fat content (13.43 %) compared to wheat flour (1.93 %) (Yalegama et al, 2013). The CF has a significantly higher fat content 25.67±1.87 % than DC treatment (Table 1). The raw material of desiccated coconut contains 68 % (SLSI, 98) of fat which is higher than the fat content of coconut flour (13.43 %). Even desiccated coconut added biscuit is expected to have higher fat content. The amount of replacement of wheat flour from desiccated coconut or defatted coconut flour is a critical factor to change the nutritional profile of biscuits.

Ash

Ash content of the food gives an overview of the mineral content. Fortification of mineral substances to the food product is a novel trend to boost the nutrient content of food while acquiring health benefits for human life. Previous studies showed that wheat flour is less in ash with the approximate figures of 0.70 % (Ocheme *et al.*, 2018). In contrast, coconut flour contains five times higher minerals (5.12 %) (Yalegama et al, 2013) than wheat flour (0.7 %) (Ocheme *et al.*, 2018). Therefore, the biscuits substituted with defatted coconut flour have a significantly higher mineral content 3.53±0.10 % (CF) than the wheat flour-based biscuits.

Crude Fiber

Crude fiber is the carbohydrate substances that our bodies cannot digest. Insoluble fiber after the digestion acts like a broom to sweep out the digestive tract while preventing constipation, infections of the gut, hemorrhoids, heart disease and some types of cancer. Therefore, consumption of fiber-rich food is very vital to boost the healthy life of a human. Defatted coconut flour contents (9.27 %) of crude fiber (Yalegama *et al.*, 2013) are higher than wheat flour (0.84 %) (Ocheme *et al.*, 2018). Therefore, significantly higher crude fiber content was observed in the CF (4.70±2.61 %) and DC (3.08±0.22 %) treatments. As crude fiber does not contribute to energy, it is expected not to contribute to a high glycemic index. So, the biscuit with high fiber content is expected to show a lower GI value.

Carbohydrate

The carbohydrate content of the sample includes the sugar and starch material which are incorporated in the food products. The sugar content of food directly contributes to the Glycemic Index of the food to categorize it as a product of low, medium or high Glycemic. Defatted coconut flour contains 46.73 % (Yalegama *et al.*, 2013) carbohydrate content which is significantly lower compared to the wheat flour having carbohydrate content of 72.73 % (Ocheme *et al.*, 2018). Results revealed that wheat flour added biscuits (WF) have the highest carbohydrate content (78.28±0.92 %) in a fresh sample. Desiccated coconut incorporated biscuit (DC) had 71.42±0.86 % carbohydrate content which is significantly lower than WF. CF has the lowest carbohydrate content (59.40 ±3.75 %) out of three types of biscuits varieties.

In-vitro measurement of different sugar fractions

The results are shown in Table 2.

Free sugar glucose content of the food product

Dietary carbohydrates are absorbed into small intestine depending on the source, physicho- chemical properties and processing aspects of the food product. Free glucose in the food is freely attached to mono and disaccharides and their alcohol substances which undergo the immediate release of glucose molecules into the intestine after the ingestion of food. A significantly higher level of free sugar glucose (sum of free glucose and glucose from sucrose) has in WF (5.88±1.03 %) than others.

Treatments	FSG	RAG	SAG	TG	HI	PGI
BR	0.45±0.17b	81.45±5.27a	59.81±7.58a	99.16±5.56a	-	-
CF	0.79±0.50b	35.10±2.96b	36.18±5.53b	40.91±1.34c	38.58	60.84
DC	0.20±0.03b	21.72±4.65c	33.97±4.82b	54.40±8.21b	45.31	64.53
WF	5.88±1.03a	38.20±5.10b	41.11±2.68b	45.84±5.60bc	42.35	62.91

Table 2. Glucose concentrations of four biscuits after in-vitro digestion

Values are Means ±standard deviations and different superscripts in the same column are significantly different ($p<0.05$), BR: White bread, CF: Defatted coconut flour-based biscuits, DC: Desiccated coconut added biscuits, WF: Wheat flour-based biscuits.

Rapidly available glucose content of the food product

Glucose which is released after 20 min of digestion helps to surge the blood sugar level after food consumption. Glucose is an important source of energy for almost all the cells in the body, especially the brain cells. Therefore, athletes may use glucose as their energy source, thus it rapidly releases the highest concentration of glucose within a shorter period of time. Results of this study revealed that significantly ($P<0.05$) higher level of rapidly available glucose (81.45±5.27 %) is released by BR within the in-vitro digestion system. Therefore, consumption of food with elevated rapidly available glucose such as white bread is not recommended for diabetic patients.

Slowly available glucose and total glucose content

Slowly releasing glucose-containing food is good for maintaining proper blood sugar status within the human system. White bread had a significantly higher slowly available glucose content of 59.81±7.58 % compared with other biscuit types. However, there were no significant effects of the incorporation of defatted coconut flour into biscuits because all the biscuit samples used for this study had similar slowly available glucose contents. However, the formula can be improved to have the desired effect.

The in-vitro system, complete hydrolysis with an excess amount of enzyme is carried out to release all the glucose to the system. Excess enzymes removed a significantly higher amount of total glucose (99.16±5.56 %) in the BR sample while lower releasing with CF (40.91±1.34 %). Therefore, these values are expected to show a lower GI value.

Hydrolysis index and predicted glycemic index of biscuits

Glycemic Index of food sample provides an overview of glucose absorption into the small intestine. The calculated hydrolysis indexes for CF, DC and WF are 38.58, 45.31 and: 42.35 respectively. When the value of the glycemic index is less than 55, it is considered as a low GI food and the value is between 55 to 70 is considered as medium GI food and more than 70 GI value is considered as a high GI food (Eleazu, 2016). The lowest PGI value has been observed in the CF sample and it has 60.84 whereas can be ranked as medium GI food. Thus, coconut flour has a substantial effect on the digestion of the in-vitro system by delaying glucose releasing to the outer environment from the food. It can be due to the significant proportion of ash and fiber content of the defatted coconut flour incorporated biscuits. The starch releasing ability of three samples and white bread revealed that the starch releasing ability of CF is low while the highest starch releasing ability in WF (Figure 1).

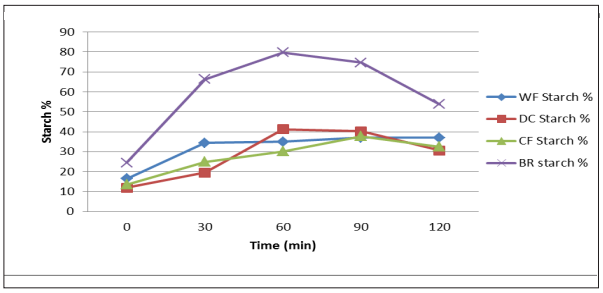

Figure 1. Starch concentration (%) of each sample during in-vitro digestionin-vitro digestion

Conclusion

Incorporation of defatted coconut flour increased the fiber and ash content of the biscuits while reducing sugar releasing capacity after in-vitro digestion. Therefore, it reduces the Glycemic Index of the product than the wheat flour-based counterparts. The defatted coconut flour-based biscuits (CF), as well as desiccated coconut incorporated (DC) and wheat flour-based biscuits (WF) can be categorized as medium GI food which is a healthy diet for a diabetic person and those who searching for healthy diets.

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Propagation and Possible Allelopathic Effects of *Vernoniazeylanica* on Selected Bioassay Species

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Abstract

Vernonia zeylanica (L.) belongs to the family Asteraceae, is one of the major endemic weed species present in coconut (*Cocos nucifera L.*) plantations of the tropics, which propagates very easily. There is a possibility that this plant could also possess allelopathic effects, but this has not been scientifically tested. Thus, a study was carried out to determine the seed germination of *V. zeylanica* under soil moisture stress conditions, shoot propagation methods and possible allopathic effects of this species, on selected species in bioassay tests. Germination of *V. zeylanica* seeds was not observed at higher osmotic potential (-0.9 MPa). The highest sprouting percentage of this species were obtained with soft wood cuttings. The aqueous leaf extract was highly phytotoxic, and it significantly reduced germination and seedling growth of all bioassay species tested. Full strength (33.3 g L^{-1}) aqueous extracts of leaves significantly reduced the germination percentage, root and hypocotyl growth rates of all species tested. The inhibitory effects were often dependent on concentration. However, the degree of inhibition varied among the test plant species. The seedling emergences of all four tested plants were severally inhibited when planted in *V. zeylanica* contaminated soil. The results indicated that incorporated aqueous leaf extract of *V. zeylanica* and its rhizosphere contaminated soil can suppress seed germination, seedling growth and seedling emergence of certain plant species indicating a possible allelopathic effect.

Key words: *Vernonia zeylanica*, allelopathic, seed germination

Introduction

Vernonia zeylanica (L.) is an herbaceous perennial deep-rooted species that belongs to the family Asteraceae and is one of the major endemic weed species present in coconut (*Cocos nucifera* L.) plantations in the tropics. It competes for soil moisture, nutrients and light especially when palms are at the seedling stage. Jayaweera (1982) stated that it is an understory shrub with many straggling, divaricated cylindrical branches when young. Therefore, it grows vigorously by covering the ground of coconut plantations under both moist and dry conditions. Additionally, it interferes and causes inconvenience to estate management practices such as manuring and harvesting of nuts. Crop growth and productivity are reduced considerably due to these factors. As this species is perennial in growth habit, it can be propagated by seeds or vegetatively by cuttings.

Allelopathy is defined as the direct influence of a chemical released from one plant on the development and growth of another plant. Allelochemicals are secreted to the rhizosphere and can suppress the growth of neighboring plants (Bais *et al.*, 2004) and might reduce the cost of weed management (Iqbal and Cheema, 2007). However, allelopathy alone might not be a perfect weed management technology; it could be a supplementary method in an integrated weed management program. A large number of allelochemicals, which are released by many weed species, have inhibitory effects on the crops (Jabeen, *et al.*, 2013). It is reported that allelochemicals which are liberated by many plants from leaves, stem, roots, fruit and seeds as residues, exudates and leachates interfere with the growth of other plants (Asgharipour and Armin, 2010). These allelochemicals are being released into the soil and show inhibitory influences on the development and growth of surrounding plants (Abdul *et al.*, 2012). However, the allelopathic effect of *Vernonia zeylanica* weed species is not fully understood.

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Therefore, the overall objectives of the present study were to determine the propagation and possible allelopathic effect of *Vernonia zeylanica* on selected bioassay species including tomato (*Lycopersicon esculentum*) chilies (*Capsicum annum* L.) radish (*Raphanus sativa* and blue rattle pod (*Crotalaria verrucose* L.).

The specific objectives of this study were to; (i) determine the effect of moisture stress on seed germination of *Vernonia zeylanica*; (ii) identify shoot propagation methods for *Vernonia zeylanica* and (iii) effects of aqueous extracts of *Vernonia zeylanica* leaves and contaminated rhizosphere soil on seed germination of selected bioassay species.

Materials and methods

The experiments were carried out in a plant house and laboratory of the Coconut Research Institute located in the Low county Intermediate Zone of the North-Western province of Sri Lanka from April to August 2016. Inside the plant house, Petri dishes and planting trays received photosynthetically active radiation (PAR) ranging from 500 – 1150 µmol m^{-2} s^{-1} and the average day and night temperature were in the range of 30-34°C and 26-30°C, respectively. Relative humidity varied between 35-60% during the day and 20-27% during the night. In the bioassay *S. lycopersicum*, *C. annum*, *R. raphanistrum* subsp. *sativus* and *C. verrucosa* were used as the test species due to their high sensitivity to the phototoxic activity of *V. zeylanicaas* observed in a preliminary study. Seeds of *V. zeylanica* were collected from five different locations in the major coconut growing regions of Sri Lanka during February and March 2016 and were stored at 5°C under dark conditions. Seeds of *L. esculentum*, *C. annum* and *R. sativa* were taken from the Seed and Plant Materials Development Center, Department of Agriculture, Sri Lanka and those of *C. verrucosa* were obtained from the same locations from which the seeds of *V. zeylanica* were gathered. Treatments of the experiments were arranged in a Complete Randomized Design (CRD) with 10 replicates (each Petri dish and a planting plot representing one replication of a single species in each trial) in the respective studies.

Effect of moisture stress on seed germination of *Vernonia zeylanica*

Aqueous solutions of polyethylene glycol (PEG)were prepared to obtain osmotic potentials of 0, -0.3, -0.4, -0.6, and -0.9 MPa by dissolving 0, 154, 191, 230 or 297 g of PEG in 1 L of deionized water (Michel and Kaufmann, 1973). Thereafter, 50 seeds of *V. zeylanica* were placed in 9 cm diameter Petri dishes containing two filter papers. The filter papers were moistened separately with 5 mL of deionized water and the respective test solutions and the Petri dishes were placed in the plant house. The germination percentage of *V. zeylanica* seeds was recorded after 30 days of incubation. This experiment was repeated three times.

Identification of suitable shoot propagation methods of *Vernonia zeylanica*

Shoots of *V. zeylanica* were collected from randomly selected *V. zeylanica* plants and they were separated into hardwood, semi hardwood and soft woods. Then the separated shoots were cut into 10 cm length. The cut surface was immediately immersed in water to avoid trapping air bubbles in the stems. The study used 200 hardwood, semi hardwood and soft wood cuttings and they were planted in a Red Yellow Podzolic soil using trays as containers. Each treatment was replicated four times and trays were kept inside the plant house. All requirements for the sprouting of different cuttings were supplied.

The data was collected as a percentage of sprouted cuttings (No. of sprouted cuttings/ Total number of cuttings) x 100.

The effect of aqueous extract of *Vernonia zeylanica* dried leaves on seed germination and seedling growth of selected bioassay species

Matured leaves of *V. zeylanica* were collected from five different locations within the major coconut growing regions of Sri Lanka from February to April 2016. The leaves were air-dried for 1 week, ground into small pieces. Different weights (33.35, 16.67 and 8.30 g) of leaf debris were put in a flask that contained 1 L of distilled water. The solutions were shaken for 12 hours at room temperature. The extract was strained through four layers of cheesecloth, then vacuum filtered through two layers of filter paper (Whatman no. 02) (White *et al.*, 1989).

Three concentrations of the aqueous extract were used, the concentrations of full-strength half strength and quarter strength were 33.35, 16.67 and 8.3 g L^{-1}, respectively (dilution was made with distilled water). Distilled water

was used as the control. The extracts were stored at 5°C until used. All the seeds of the test plant species were surface-sterilized for 1 minute in a 50% sodium hypochlorite solution, rinsed with running water for 10 minutes, and air-dried. Fifty seeds from each selected bioassay species were placed separately in 9 cm diameter Petri dishes lined with cotton wool. The solutions and distilled water were applied in 5 mL volumes per dish. The Petri dishes were kept in the plant house for 72 hours at 28– 30°C. All treatments were replicated 10 times. Seed germination percentage, root and hypocotyl length of the selected bioassay species were measured 3 weeks after seed placement (White *et al.*, 1989).

Effect of residual toxicity of contaminated soil on seed germination of bioassay species

Contaminated soil was collected to a depth of 10 cm from a field where *V. zeylanica* had been growing for the last 5 years. Soil from a field where *V. zeylanica* had not been growing was used as a control. Soil samples were air-dried and sieved through a 2 mm mesh. Ten g were taken from each soil sample and uniformly spread individually in 9 cm diameter Petri dishes. Fifty seeds of the selected bioassay species were placed uniformly on the soil and covered with a further 10 g of the same soil. The soil was moistened to field capacity with distilled water. The dishes were kept in a plant house at 27-30°C. Each treatment was replicated 10 times.

Statistical analysis

An Analysis of Variance (ANOVA) using the statistical software SAS was carried out on the data from all experiments and the significance of observed differences was tested using Least Significant Differences (LSD) at 5% probability (SAS Institute 1999).

Results and Discussion

Impact of moisture stress on seed germination of *Vernonia zeylanica*

Germination of *V. zeylanica* seeds decreased when the osmotic potential increased from 0 MPa to -0.9 MPa (Table 1). Application of higher osmotic potentials (-0.9 MPa) did not facilitate germination while the highest germination percentages (12.4, 34.8 and 42.8%) were found in the control treatment (0 MPa). Treatment 2 and 3 (T_2 and T_3) induced seed germination but the effect of these two treatments on seed germination was not significantly different. However, no seed germination was observed in the high moisture stress conditions at -0.9 MPa. The results suggested that under higher moisture stress conditions, germination of *V. zeylanica* seeds was suppressed.

Treatments	Seed germination %		
	1st round	2nd round	3rd round
T_1 Control	12.4	34.8	38.8
T_2(– 0.3 MPa)	10.8	22.6	24.5
T_3(– 0.4 MPa)	10.8	18.3	20.4
T_4(– 0.6 MPa)	0.0	5.5	4.2
T_5(– 0.9 MPa)	0.0	0.0	0.0
Signifiance	*	*	*
LSD (P = 0.05)	6.51	10.6	10.8

Table 1. Effect of soil moisture stress (osmotic potential –MPa) on seed germination percent of *Vernonia zeylanica*

Moreno and McCarthy (1994) found that crabgrass (*Digitaria sp.*) germination was reduced by up to 70% at osmotic potentials ranging from -0.4 to -0.8 MPa. The germination of the sensitive plant (*Mimosa pudica* L.) and Caesarweed (*Urena lobata* L.) was sensitive to simulated water stress and less than 12% of the *M. pudica* and *U. lobata* seeds germinated at osmotic potential below -0.4 MPa (Senarathne and Sangakkara, 2010). This weed species seems to be best adapted to germinate in a moist environment, and germination in the field may depend on adequate water availability.

Shoot propagation methods of *Vernonia zeylanica*

A significantly higher sprouting percentage was observed in soft wood cuttings (T_3) when compared to hardwood (T_1) and semi hardwood cuttings (T_2) (Table 2). The highest sprouting percentage (89%) was found in soft wood cuttings at 12 days after planting, and the sprouting percentage in hard wood cuttings in the same period was 30.5%. However, the highest sprouting percentage (36%) of hard wood cuttings was observed 15 days after planting.

Treatments	Percentage of sprouting			
	6 days after planting	9 days after planting	12 days after planting	15 days after planting
T_1 Hardwood	12.4	34.8	38.8	36.0
T_2-Semi hardwood	10.8	22.6	24.5	61.0
T_3-Softwood	10.8	18.3	20.4	85.0
Signifiance	*	*	*	*
LSD (P = 0.05)	6.51	10.6	10.8	18.81

Table 2. Effect of shoot maturity on sprouting of *Vernonia zeylanica* cuttings

Treatments (g dried leaf L^{-1})	Seed germination (%)			
	Lycoper-sicon esculentum	*Capsicum annum*	*Raphanus sativa*	*Crotalaria verrucosa*
T_1 Control (Water)	70.8	76.0	82.8	83.2
T_2 8.3	45.6	63.6	61.6	69.2
T_3 16.7	24.0	47.6	47.6	51.6
T_4 33.3	10.4	26.8	26.8	27.6
Signifiance	*	*	*	*
LSD (P = 0.05)	9.01	5.14	6.83	10.35

* Significant

Table 3. Effect of aqueous extracts of dried leaves on seed germination of bioassay species

The sprouting percentage of hard wood, semi hard wood and soft wood cutting 6 days after planting were 25.5, 50.0 and 75.4%, respectively. Except in T_3, the percentage of sprouting was increased with the increasing number of days after planting.

The effect of aqueous extract of *Vernonia zeylanica* dried leaves on seed germination and seedling growth of selected bioassay species

Aqueous extracts from dry leaves of *V. zeylanica* reduced the germination percentages of *L. esculentum*, *C. annum*, *R. sativa* and *C. verrucosa* when compared to the control treatment (Table 3).

The lowest seed germination (10.4%) was found in *L. esculentum* seeds at the highest concentration of aqueous extract of *V. zeylanica* (T_4) whilst the highest germination percentage (83.2%) was recorded in *C. verrucosa* seeds in the control treatment (T_1). The inhibitory effect of *V. zeylanica* leaf aqueous extract on seed germination of the above bioassay species could be due to an allelopathic effect. The overall results suggested that the leaf aqueous extract of *V. zeylanica* (P> = 0.05) suppressed the seed germination of *L. esculentum*, *C. annum*, *R. sativa* and *C. averrucosa*. These results are supported by the findings of Mishra and Singh in 2010, who reported that aqueous leaf extract of Lantana camara produced an inhibitory effect on the growth of *Parthenium hysterophorus* and caused a significant inhibitory effect on seed germination, root and shoot elongation and development of lateral roots of six popular agricultural crops.

Effect of the aqueous extract of dried leaves on the root elongation of the bioassay species

Root growth of the weeds was significantly reduced by the different concentrations of aqueous extracts of *V. zeylanica*. Results have shown that the inhibitory effects of the aqueous extracts increased with their concentrations. However, variations in the response were observed in the selected bioassays, where *C. verrucosa* produced the highest and *L. esculentum* the lowest root length under the influence of the aqueous extracts.

At the highest concentration (33.35 g L^{-1}), *L. esculentum*, *C. annum*, *R. sativa* and *C. verrucosa* root growth was suppressed by 100, 87.4, 100 and 72.9% respectively, when compared to that of plants in the control treatment (Figure 1). At quarter-strength (8.3 g L^{-1}) the root length of *L. esculentum*, *C. annum*, *R. sativa* and *C. verrucosa* decreased by 85.0, 61.2, 80.0 and 32.7%, respectively compared to root growth of control. Aqueous extracts of some weed species leaves have a negative impact on weed seedling growth through allelopathy. The reduction in weed seedling root length may be attributed to the reduced rate of cell division and cell elongation related to the presence of allelochemicals in the extracts (Fariba *et al.*, 2007).

Effect of the aqueous extract of leaves on the hypocotyl elongation of the bioassay species

The aqueous extract was either inhibitory or stimulatory to the hypocotyls growth of selected bioassay species depending on the

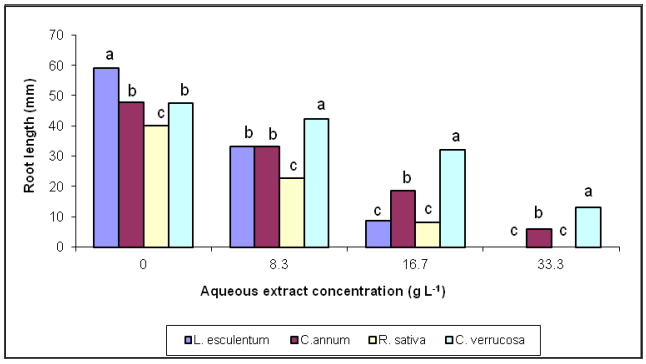

Figure 1. Changes of the root length of the selected bioassay seedlings in response to different concentration of the aqueous extract of *Vernonia zeylanica*
(Bars with the same letter are not significantly different within extract concentration P=0.05)

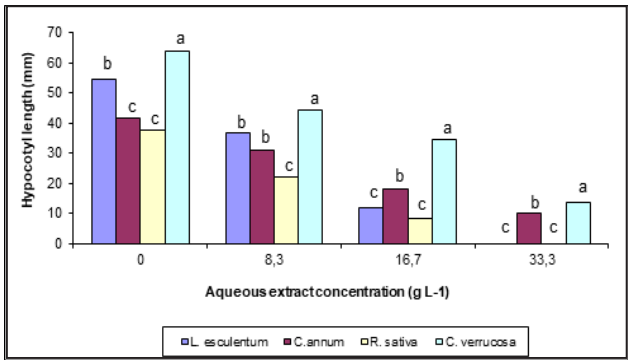

Figure 2. Changes of the length of the hypocotyl of the selected bioassay seedlings in response to different concentration of the aqueous extract of *Vernonia zeylanica*
(Bars with the same letter are not significantly different within extract concentration P=0.05)

extract concentration and the plant species. The application of the aqueous extract at full-strength resulted in complete inhibition of *L. esculentum*, and *R. sativa* growth. At this concentration, hypocotyl elongation of *C. annum* and *C. verrucosa* hypocotyls was suppressed by 76.3 and 78.6%, respectively compared to the control plants. At quarter-strength, the hypocotyls growth of all the selected bioassay was reduced by 78.4, 56.3, 78.0 and 46.1%, respectively, when compared to those of the control treatment.

In response to the half-strength extract, the hypocotyl lengths of all the selected bioassay species were decreased by 33.0, 24.8, 40.7 and 30.9% respectively, while *L. esculentum* and *C. annum* did not show any significant change in comparison to the control treatment (Figure 2).

Effect of residual toxicity of contaminated soil on seed germination of bioassay species

Soil collected from the *V. zeylanic* arhizosphere had a strong inhibitory effect on the seed germination of the bioassay species *L. esculentem*, *R. sativa* and *C. annum* (Figure 3). However, the allelopathic effect of *V. zeylanica* on *C. verrucosa* seeds was not significant. The lowest germination percentage (32.8%) was recorded in *L. esculentem* seeds, when these seeds were sown on the *V. zeylanica* contaminated soil, while the highest germination percentage was found in *C. verrucosa* seeds (56.3%), compared to the germination percentage of other selected bioassays (Figure 3). This agrees with the results of Chung and Miller (1995) who reported the inhibitory effect of soil collected from the area

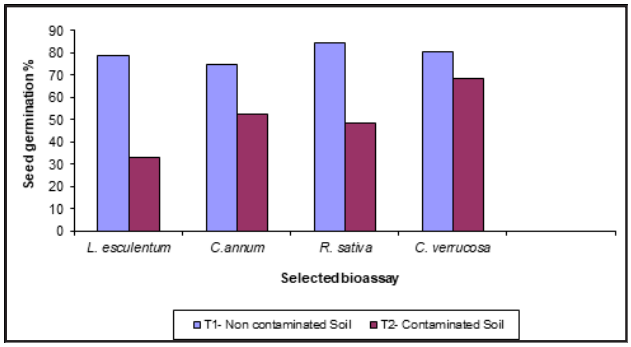

Figure 3. Effect of rhizosphere soil of *Vernonia zeylanica* on the seed germination percentage of selected bioassay species

surrounding alfalfa (*Medicago sativa* L.) plants on bioassay test species. This inhibition may be due to the release of phytotoxic substances by the root itself or through interaction between microorganisms and tissue litter. However, this interpretation needs further study as several factors are involved in an allelopathic activity and seed germination. Besides, allelochemicals released into the rhizosphere exert a significant impact on nutrient availability, dynamics and uptake by the plant. A broader knowledge of the effects of plant allelochemicals on mineral nutrient soil cycles, heavy metal detoxification and nutrient solubility can enhance the nutrient use efficiency through a reduction of their losses and the development of a more efficient and sustainable fertilization technique (Aurelio *et al.*, 2019).

Conclusions

According to the given results, it was suggested that under higher moisture stress conditions germination of *V. zeylanica* seeds was suppressed and soft wood cuttings have the highest sprouting percentage of *V. zeylanica*.

The selected bioassay species were more sensitive to the inhibitory effects of aqueous extracts and contaminated rhizosphere soil of *V. zeylanica*. Hence, it can be suggested that *V. zeylanica* has a possible allelopathic potential and releases allelopathic substances to the environment. However, the species sensitivity to allelochemicals and extent of inhibition varies between species. The allelopathic effect of *V. zeylanica* may be an important mechanism involved in the invasive success of this plant. Under natural conditions, where a great number of interactions with other organisms occur, these allelopathic effects can enhance or restrain plant growth and species diversity. Field experiments must be carried out to test the effectiveness of the allelopathic potential of the above species under natural conditions.

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Guidelines to the Contributors of CORD

The International Coconut Community (ICC) publishes annually an international journal on coconut research and development known as *Cord*. High standard original articles written in English are published in *Cord*. The manuscript, complete in all respects, should be submitted to www.journal.coconutcommunity.org by register first. The high resolution scanned tables, photographs, graphs, etc may be sent to journal@coconutcommunity.org for easy editing.

Hereunder are the guidelines/editorial style in preparing the article for CORD:

Type the manuscript in A4 size paper with double line spacing using MS word. **The title** of the article should be short and specific. It should be typed in bold letters, for scientific name use *italics*.

Below the title, type **author's** (s) **name** (s), each name should be superscripted as 1, 2 …. Each superscripted number should be explained by providing the complete **mailing address** of the author (s) with all details including pin code, e-mail etc at the bottom of first page.

Below the author (s') name (s), provide an **abstract** emphasizing the key aspects of results but without reference to the body of the text of the full articles with the maximum of **250 words**. After the abstract, provide a list of the **Keywords**: (4-6).

The introduction should have a brief statement of the problem and explain the aim or the objectives of the investigation. **The materials and methods** should be very clear with all details of experimental design, treatments, location, period of study, methods adopted etc. Methods should be clearly written so that the reader of your article should be able to use it in pursuing her/his studies elsewhere.

The results and discussions should provide data organized into tables, figures and photographs, suitably compared and discussed with earlier published findings. All data must be presented in metric units.

For abbreviations, consult the B.S. (British Standards) on "Letter symbols, signs, and abbreviations". Abbreviations are alike in singular and plural. The placing of a dot (stop) after a standard abbreviation should be avoided unless the abbreviation form is likely to be taken for another word, e.g., in for inch, where a full stop would be necessary.

In the text, references should be quoted according to the Harvard system, in which the author's name and date are given in parenthesis, e.g. (Magat, 2003) except when the author's name is part of a sentence, e.g. Magat (2003) reported …. When there are two authors, both names should be given, e.g. (Magat and Margate, 1990). If there are more than two authors, give the first author's name only followed by the words *et al*. e.g. Magat *et al.* (1989).

The **table** should carry appropriate titles, **which should be typed in bold letters**. Each table should be self-explanatory, numbered consecutively. Try to avoid presenting table that is too large to print across the page. Use - (dash) when no observation was taken and 0 for zero reading. Express values less than unity as 0.25 and not .25.

The **figures should** be restricted to the display of results when large number of values can be comprehended more easily; tables and figures should not reproduce the same data. Numbering and lettering should be kept to an absolute minimum. The **photos and plates** should be of high quality and be able to make a definite contribution to the value of the paper. The **acknowledgment** should follow the section on discussion and immediately precede references. Only **references** quoted in the text should be listed. References in the text should be arranged chronologically.

Reference to publications in periodicals should be cited in the following order surname of author, followed by the initials, year of publication, title of paper, name of journal (italics), volume (in bold Arabic letters), and number in bracket and after a colon the first and last pages of the reference (without the prefix "pp.") e.g. Morin, J.P, Sudharto, P.S., Purba, R, Desmier de Chenon, Kakul, T., Laup, S., Beaudoin-Ollivier, L. and Rochat, D. 2001. A new type of trap for capturing *Oryctes rhinoceros* (Scarabaeidae: Dynastinae), the main pest in young oil palm and coconut plantings. *Cord* **17**(2): 13-21.

Reference to books and monographs should include author and/or editor's name full title, number of pages, edition, the publisher's name and place of publication. The title should be in italics. A list of references should be given at the end of the text and be arranged alphabetically on authors' name. If an author's name in the list is also mentioned with co-authors the following order should be used: publications of the single author, arranged according to publications dates, publications of the same author with one co-author and publications of the author with more than one co-author. If there are several references by the same author in one year, they are distinguished by the addition of small letters a, b, c, etc., e.g. Magat, 2003a, Magat, 2003b.

Abbreviations of journal titles should be according to the "World list of Scientific Periodicals."

Note: The author(s) of selected paper will receive a token honorarium, as well as one-year free subscription of *Cord* for senior author if there is more than one author. Articles not accepted will not be sent back to the author.

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