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Analysis of Soot Suppressing Effect of Bio-Diesel in a Compression Ignition Internal Combustion Engine

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Abstract *– Out of many pollutants, diesel particulates (soot) play a major role when it comes to emission controlling in the transport sector. Fine particles of soot can irritate various respiratory and cardiovascular health problems to human beings.*

The addition of oxygenated fuels has taken a significant attention to reduce harmful emissions from petroleum-based fuels. The objective of the current study was to investigate the effect on soot formation of biodiesel produced for this experimental study.

The contribution of different hydrocarbon classes on soot formation, soot formation models and the contribution of hydrocarbon saturation on soot formation were studied. Hydrocarbons with higher amount of saturation has a higher tendency to form soot. For the current study, coconut oil was used to produce biodiesel. Out of many vegetable oils, coconut oil has the lowest concentration of unsaturated hydrocarbons. Biodiesel has a lower calorific value but a higher cetane index compared to that of diesel fuel.
The experiment was carried out on a vehicle fitted with diesel engine. A separate opacity meter was used to measure the soot concentration of diesel emissions.
Tests were carried out with 11 fuel samples which included 100% diesel fuel, 100% biodiesel samples and nine other blends of biodiesel in diesel fuel with compositions varying between 90 % (10% biodiesel) and 10% diesel fuel (90% biodiesel).

During the experimental investigation, the lowest soot concentration was observed when the vehicle was fueled with 100% biodiesel and the highest amount was observed when the vehicle was fueled with pure diesel fuel. At higher concentration of biodiesel blends, a significant reduction of soot concentration was observed. At low concentrations of biodiesel, the reduction of soot concentration was not significant. The results obtained during the research are in comparison with the outcome of previous research.

Keywords: *soot, soot formation, oxygenated fuels, bio diesel*

1 INTRODUCTION

Despite advancements made with respect to electricity, Hydrogen and other alternatives such as Bio Diesel, fossil fuels are very likely to be the main supplier of energy for the transportation system for the next 4 to 5 decades (Lei Xu et.al., 2022).

It is well known that fossil fuel used in the transport sector emit much of the harmful emissions to the atmosphere. Out of many harmful emissions, diesel particulates play a major role when it comes to emission controlling in the transport sector.

Due to the absence of a throttle body by nature, compression ignition engines operate in a lean region of air fuel ratio. However, even if the fuel is burned in the presence of more than enough oxygen, soot may form due to poor fuel quality, fuel system component malfunction or non availability of time to complete the ignition etc.

Diesel particulates from automobiles powered by CI Engines results in direct exposure to human beings. Especially, the fine particles of soot can have various respiratory and cardiovascular health problems in human beings (Lighty et.al., 2011).

Although the total oxygen content within the combustion chamber of a diesel engine may be sufficient for complete combustion, due to uneven mixture formation, localized rich mixtures exist (see Fig. 1).

If it is assumed that a four stroke engine is operating at 3000 rev/min, and diesel fuel is injected 20^0 degrees before TDC and combustion process continue further 20^0 after TDC, only 22 ms is available for the whole process to occur.

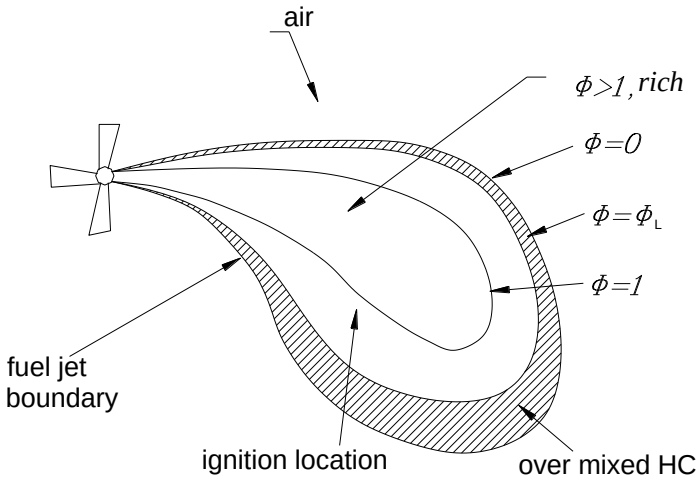

Fig. 1 - Equivalence ratio distribution at the beginning of combustion (Heywood 1981)

The ignition starts at a point in the slightly lean region downstream of the spray core, where the fuel air mixture has spent more time in the combustion chamber. In the mixing mechanism, the degree of mixing of fuel with air varies with equivalence ratio, Φ, and this value reaches the combustible limit Φ_L and approaches a limit of zero. At the middle of the spray core is a rich mixture of fuel.

In order to reduce the harmful effects of soot produced by diesel fuel combustion, various alternatives such as after treatment, modifications to the fuel injection system, fuel additives have been proposed. Out of many alternatives the addition of oxygenated fuels also has taken a significant attention. Fuels such as alcohols, vegetable oil, and bio diesel are considered as alternatives for oxygenated fuels (Shigeru Tosaka and Yasuhiro Fujiwara., 2000). The objective of the current study is to investigate the effect on soot formation of biodiesel produced for this experimental study.

2 EFFECT OF FUEL STRUCTURE ON SOOT FORMATION

Soot is formed from unburned fuel, which nucleates from the vapor phase to a solid

phase in fuel-rich regions at high temperatures. Out of the major hydrocarbon classes, unsaturated hydrocarbons, aromatics and polycyclic aromatic hydrocarbons have the greatest influence on soot formation. Shigeru Tosaka and Yasuhiro Fujiwara (2000), have carried out a series of tests to investigate the soot formation mechanisms of different hydrocarbon classes. It was revealed by Shigeru Tosaka and Yasuhiro Fujiwara, (2000) that different hydrocarbon classes start to form soot at different temperatures. Benzene starts to form soot at the lowest temperature and is increased in the order 1-hexane, cyclohexane and n-hexane. The fuel which starts to form particulate matter at lower temperatures emits higher amounts of particulate matter. It was further observed that benzene does not decompose and formation of soot took place through polymerisation and formation of polycyclic aromatic hydrocarbons.

Tao et. al. (2009) have explained in detail and validated a soot formation model. According to Tao et. al. the soot formation process can be explained in nine steps as shown in Fig. 2.

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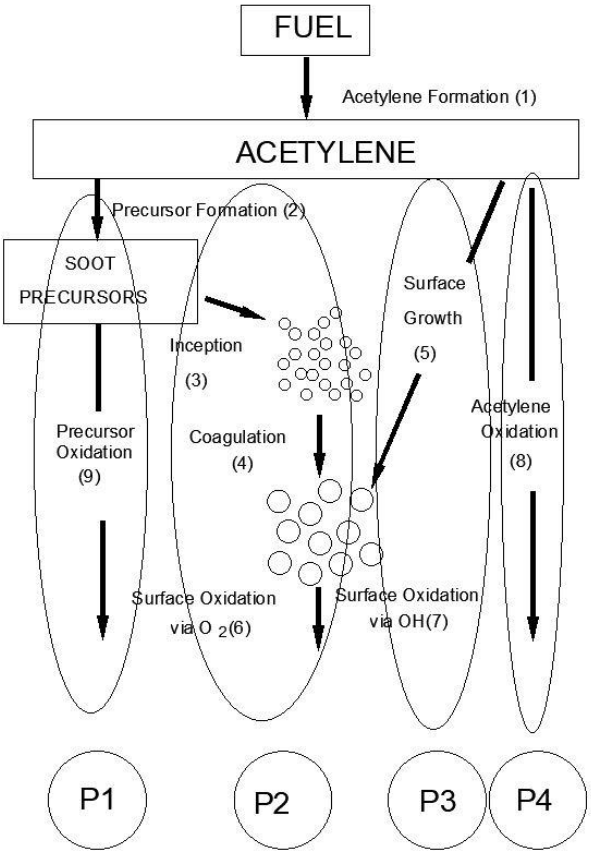

Fig. 2 - Soot formation model [Tao et.al (2009)]

According to Tao et. al. there are four main paths (P1- P4) for soot formation and the first step is fuel pyrolysis leading to the formation of acetylene which is a gas phase soot precursor. According to the model, soot can be formed by formation of precursors and soot formation by precursor oxidation (P 1).

The second path (P2) is via process 2, 3, 4 and 6 where, acetylene is formed via pyrolysis and soot precursors are formed. These soot precursors form young soot particles that

contain significant amount of carbon.

The developing soot particles are assumed to have a diameter of 1.28 nm, which corresponds to about 100 carbon atoms. These developing (budding) particles are coagulated and oxidized via OH to form final soot particles.
The third path (P3) is by formation of acetylene and direct surface growth and coagulation to form soot. The fourth path (P4) is direct acetylene oxidation.

Regardless of the path that the fuel particle follows, the general process can be summarised as:

a) formation of gas-phase soot precursors,

b) soot nucleation,

c) surface growth and coagulations,

d) soot oxidation.

According to the model, the local deficiency of oxygen favours the soot formation. Formation of soot precursors, inception and surface growth of soot is accommodated because of the deficiency of local oxygen. Based on the argument that soot is formed due to lack of local oxygen within the fuel core, the effect of oxygenated fuels (biodiesel) is investigated.

The formation of gas phase precursors involves the formation of Acetylene and involves the well-known hydrogen-abstraction–carbon-addition reactions (Zhang., 2016). The main soot precursors are considered to be acetylene, benzene and un-saturated Polycyclic Aromatic Hydrocarbons.

Although, the latest research has been able to elaborate more on the details, the above model remains valid up-to-date (Lei Xu et.al., 2022).

3 PRODUCTION OF BIODIESEL

All naturally existing fats and oils are esters of glycerol, consisting of three long-chain fatty acids that are bonded to a single glycerol molecule. With few exceptions, fatty acids, from which the fats and oils are derived, are straight chain compounds ranging in size from three to eighteen carbon atoms.

Since three long chain fatty acids are bonded together to form a glycerol molecule, such a compound is called a triglyceride. Triglycerides are heavy in molecular weight and low in cetane number compared to that of average diesel hydrocarbon. The length of carbon chains, as well as the number, orientation, and position of double bonds in these chains vary from one triglyceride to another. If the triglycerides are cracked and separated from the glycerol molecule by a suitable method, a hydrocarbon with the carbon number, which is almost equivalent to Cetane (hexa decane), can be obtained.

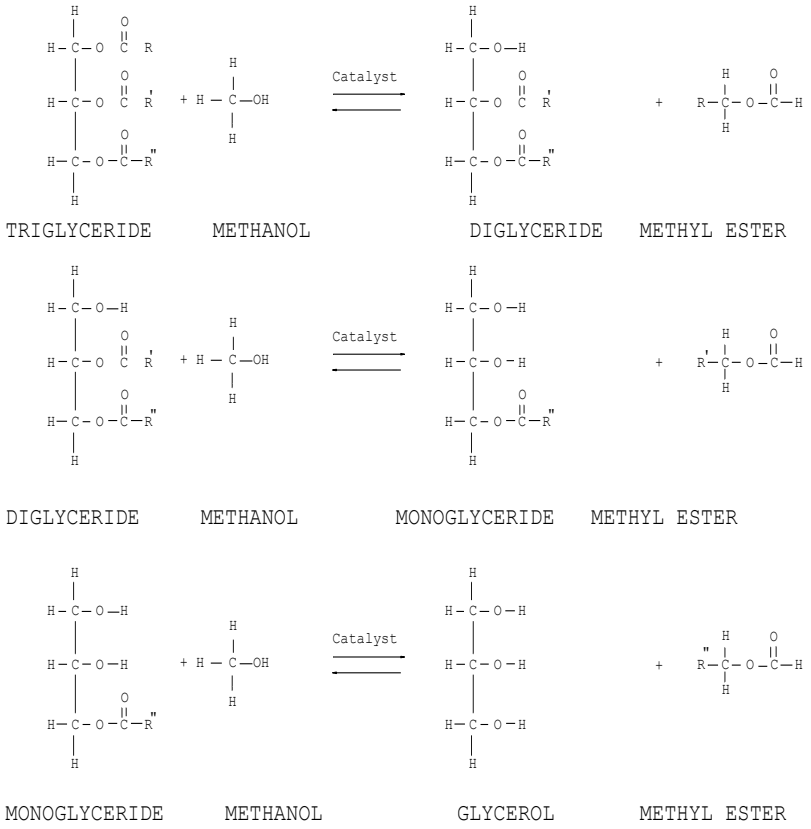

Fig. 3 - The process of biodiesel preparation

The bio diesel is prepared by breaking fatty acids from the glycerol molecule and joining an alcohol to the fatty acid as shown in Fig. 3. First the tri-glyceride is converted to a dy-glyceride by releasing a fatty acid molecule from the triglyceride to form a fatty acid ester. Then the dy-glyceride is converted to a mono-glyceride by liberating another fatty acid molecule. Finally, the mono-glyceride is converted to glycerol by liberating the third fatty acid molecule.

A catalyst is used to improve the reaction rate. Since the reaction is reversible, excess alcohol is used to shift the reaction to obtain the maximum yield of biodiesel.

After trans esterification, the mixture separates into fatty-acid methyl ester and glycerol (see Fig. 4). The fatty acid methyl ester is separated from glycerol by gravity and is washed. The neutral fatty-acid ester is called “biodiesel”.

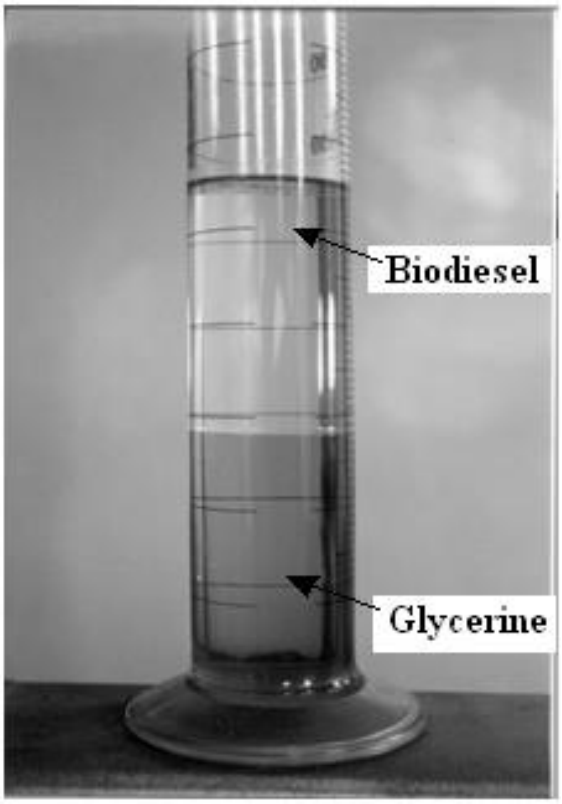

Fig. 4 – Separation of biodiesel

For the current study, coconut oil has been used with methyl alcohol to produce biodiesel. Biodiesel is an oxygenated fuel with a higher cetane number. The conversion of triglycerides into methyl or ethyl esters through the transesterification process reduces the molecular weight to one-third that of the triglyceride. Further, this reaction reduces the viscosity of vegetable oil and the final viscosity is almost similar to that of diesel fuel. These esters contain 10 to 11% oxygen by weight, which may encourage complete combustion than hydrocarbon-based diesel fuels in an engine.

Table 1 – Physical and Chemical Properties of Biodiesel and Diesel Fuel

Property	Diesel fuel	Biodiesel
Viscosity (cSt)	2.82	4.2
Flashpoint (^{0}C)	83	113
Calorific value (kJ/kg)	45, 000	38,000
Cetane Index	40	60

The cetane number of biodiesel is around 60. Biodiesel has volumetric heating values about 12% less than diesel fuels, but has a high cetane number and high flash point. A comparison of physical properties of biodiesel and diesel fuel is given in table 1.

From the review on soot formation, two main factors contributing to the soot formation can be identified. (a) Polycyclic aromatic hydro carbons act as precursors for soot formation (b) Soot is formed in the fuel rich region of the fuel spray.

If biodiesel is used as a fuel or mixed with diesel fuel, major portion of fuel will consist of straight chain paraffin hydro carbons and this will reduce the tendency to form soot as the precursors are less. Further, biodiesel contain 10% - 11% Oxygen by mass which can help the combustion process within the fuel rich region.

4. FATTY ACID COMPOSITION ON SOOT FORMATION

Biodiesel is made from natural feedstock which consists of three long chain fatty acids that are bonded together to form a glycerol molecule. The hydrocarbon chain length of the fatty acid and the degree of saturation depends on the raw material used.

Table 2 - The composition, total saturated and total un saturated fatty acids by weight for some of the vegetable oils (Folayan 2019).

Fatty Acid	Formulae	Coconut oil	Palm oil	Soybean oil	Corn oil	Olive oil	Canola oil
Saturated components							
Caproic	C6H12O2	0.59	0.4	-	-	-	-
Caprylic	C8H16O2	8.1	3.8	-	-	-	-
Capric	C10H20O2	6.5	4	-	-	-	-
Lauric	C12H24O2	47	49.5	0.1	–	-	-
Myristic	C14H28O2	18.6	14.9	0.2	0.17	0.01	0.18
Palmitic	C16H32O2	8.4	7.8	10.5	12.1	13.6	4.35
Stearic	C18H36O2	2.6	2.5	3.8	2.3	2.7	2
Arachidic	C20H40O2	0.1	0.1	0.3	–	0.5	0.5
Behenic	C22H44O2	-	-	0.28	–	0.12	–
Ligoceric	C24H48O2	-	-	-	-	0.6	–
Total saturated		91.89	83	15.18	14.57	17.53	7.03
Unsaturated							
Palmitoleic	C16H30O2	-	-	–	0.12	1.65	0.275
Oleic	C18H34O2	6.4	14.7	23.7	30.9	68.2	59.4
Linoleic	C18H32O2	1.6	2.2	54.5	53.3	11.5	21.15
Linolenic	C18H30O2	0.1	–	6.3	1.1	0.9	10.35
Eicosenoic	C20H38O2	-	-	-	-	0.2	0.8
Erucic	C22H42O2	-	-	0.25	–	–	0.78
Total unsaturated		8.1	16.9	84.75	85.42	82.45	92.75

Wang et.al., (2016) have also observed that Biodiesels produce lower soot compared to diesel fuel because of the oxygen atoms in the fuel. Further Wang et. al., (2016) have observed that during the combustion, higher saturated biodiesel fuel produces less acetylene and soot precursors than un-saturated biodiesel and the acetylene production is proportional to the number of carbon–carbon double bonds in the Fatty Acid Methyl Ester structure. The net soot production is the result of the combined effect of acetylene and precursor species formation. Biodiesel fuels with a lower fraction of unsaturated Fatty Acid Methyl Esters result in lower soot emissions. Sarathy et.al., (2007) have concluded that unsaturated Fatty Acid Methyl Esters would have a greater tendency to soot than a saturated Fatty Acid Methyl Esters.
The fatty acid, formulae and the composition total saturated and total un-saturated fatty acids by weight for some of the commonly used vegetable oils to produce biodiesel is given in Table 2.

Out of the six oils compared in table 2, Canola oil has the highest un saturated content of 92.75% by mass and the lowest unsaturated content is for Coconut oil. The total unsaturated hydrocarbon content of coconut oil is 8.1% by mass. As it was observed by other researchers that lower the unsaturated fat, lower the soot concentration. It is expected that biodiesel produced from coconut oil would produce less amount of soot.

5. PROCEDURE

The experiment was carried out on a vehicle fitted with an engine of specifications given in Table 3. A separate MAHA LPS 200 opacity meter was used to measure the soot concentration of diesel emissions. The measurement of the opacity meter is based on the amount of light extincted during its travel when a gas containing certain amount of soot particles (Wang et.al., 2016). The smoke density is expressed on a per meter basis (m^{-1}) also known as the K factor. The zero and full scale readings of the smoke meter has been calibrated to display 0.0 ± 1.0% and 100.0 ± 1.0%. Optical sensors were cleaned using purge air system of the opacity meter as per the manufacturer recommendation. Each test was repeated three times and average was taken. If the maximum deviation between three readings was more than 5%, the test was repeated. During operation, the accelerator pedal was moved to the fully open position as rapidly and held until the engine reaches tje maximum speed and the emission meter take a reading.

Table 3 -Test Engine Specifications

Type of engine	Four cylinder four stroke
Bore diameter	86 mm
Stroke	85 mm
Total displacement	1974 cm^3
Compression ratio	23:1
Turbocharger	None
Rated power	55 kW @ 4700 rpm
Peak torque	97 Nm@ 2600 rpm

Prior to the investigation, the injector pump was serviced and calibrated to manufacturers' specifications. A new set of injectors with correct injection pressure was fitted. In order to ensure that all fuel sediments were expelled from the system, the fuel system was completely flushed out before the engine was charged with a new fuel blend. The filter too was replaced for the same reason with the same frequency. Tests were carried out with 11 fuel samples which included 100% diesel fuel, 100% biodiesel samples and nine other blends of biodiesel in diesel fuel with compositions varying between 90 % (10% biodiesel) and 10% diesel fuel (90% biodiesel). The blend reference number and composition are given in table 4.

Table 4- Blends of fuels used for the experiment

Blend reference number	1	2	3	4	5	6	7	8	9	10	11
Diesel composition	100	90	80	70	60	50	40	30	20	10	0
Bio diesel composition	0	10	20	30	40	50	60	70	80	90	100

6. RESULTS

The engine was started and warmed up to the operating temperature. The K factor was measured for different fuel blends starting from blend 1 to blend 11 at full throttle position of the engine at snap acceleration as per the SAE J1667 standard test procedure (SAE J1667., 1996). The K factor for various biodiesel blends with diesel fuel was measured. Fig. 5 depicts the variation of opacity for various fuel blends.

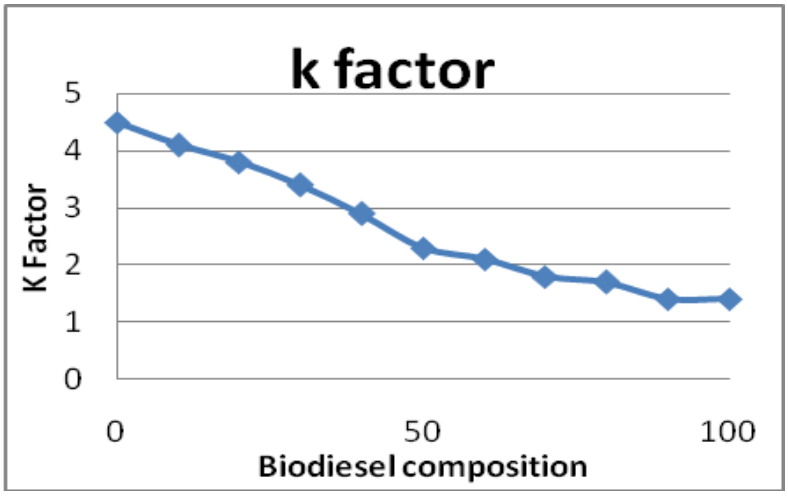

Fig. 5 - Soot Level for Various Biodiesel Blends with Diesel Fuel

From Fig.5, it is clear that the soot emission level of the engine has reduced with the increase of biodiesel concentration in the fuel. The lowest level of soot is recorded for 100% biodiesel. The K factor for diesel fuel was 4.5 and it has reduced to 1.4 when the test was carried out for pure biodiesel. When the engine was fuelled with 100% biodiesel, the soot level was reduced by 3.2 times compared to the soot concentration for 100% diesel fuel.

7. **DISCUSSION**

Biodiesel contains 10%-12% oxygen by mass and therefore it is a good oxygenator, which may help to burn hydrocarbons especially in fuel rich region of the diesel spray. Since the cetane number is high for biodiesel compared to that of diesel fuel, the ignition delay period is shorter for biodiesel, which results in less soot emissions. Absence of aromatic hydrocarbon and polycyclic aromatic hydrocarbon classes in biodiesel is the greatest advantage. Since aromatic and polycyclic aromatic hydrocarbons are acted as precursors for soot in flames, biodiesel does not provide necessary precursors for the process of soot formation. Low concentration of soot have been observed by Schobing et.al., (2018) when the biodiesel concentration was increased. Further they have observed that high concentration of bio diesel in diesel fuel have increased the oxygen concentration and ash content in soot. They have attributed this phenomena to better oxidation of soot with the increased amounts of biodiesel. Abbouda et.al., (2018) have carried out Soot volume fraction measurements for three different methyl ester compounds as Methyl butanoate, methyloctanoate and methyldecanoate. They also have observed a reduction in soot concentration with the increase of Biodiesel concentration. Further, they have observed a decreasing carbon formation with decreasing the carbon chain length of methyl ester.
Tian et. Al. (2019) have applied Extinction calibrated laser induced incandescence (LII) to measure the soot volume fraction in laminar pool fires. They have observed that the peak soot volume fraction produced by neat biofuels are 10.6 to 32.6% that of diesel fuel. Further, they have observed slower reduction in total soot amount for blending of small quantities of biodiesel and faster decrease towards neat biodiesel.

8. **CONCLUSION**

It was revealed in the experimental investigation that biodiesel has a significant soot suppressing effect and biodiesel can be used as a soot inhibitor.
During the experimental investigation, the soot concentration varied between 4.5 and 1.3. The lowest soot concentration was observed when the vehicle was fueled with 100% bio diesel (blend 11) and the highest amount was observed when the vehicle was fueled with pure diesel fuel (blend 1). The difference is a 71,1% reduction in soot concentration from that of pure diesel fuel. At higher concentration of biodiesel blends such as 90%(blend 10) - 70% (blend 8), the soot concentration remained below 2 and the percentage reduction of soot concentration was more than 57% of that for pure diesel fuel. At low concentrations of biodiesel, the reduction of soot concentration was not significant. When the biodiesel concentration varied from 10% – 30% (blend 2 and blend 4), the soot concentration varied between 4.1 and 3.4 which is only a 17% reduction from that of the pure diesel fuel. The results obtained during the research are in comparison with the outcome of other researchers.

7. ACKNOWLEDGEMENT

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In vitro Antifungal Properties of the Different Solvent Extracts of Selected Tropical Plants

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Abstract - *Several species of fungi are responsible for the post-harvest deterioration. Among them, Rhizopus stolonifer, Colletotrichum gloeosporioides and Aspergillus spp. are the major causal organisms of post-harvest diseases. Synthetic fungicides are one of the most effective controlling methods for health hazards. Hence, there is an urgent need for developing a bio-safe method for the control of this pathogen without the use of chemical fungicides. The aim of the present study was to investigate* the *Antifungal effect of Adhathoda vasica - leaves, Azadirachta indica- seeds, Ricinus communis - seeds, Clerodendrum infortunatum-leaves and Pistia stratiotes- leaves via in vitro, on the growth of Rhizopus stolonifer, Colletotrichum gloeosporioides* and *Aspergillus spp. Five different solvents; methanol, ethanol, chloroform, petroleum ether, and sterilized distilled water were used for plant extraction. Disk diffusion assay was conducted to evaluate the performances of each plant extract against all three fungi. A wide range of yields among extracts was observed depending on the extraction solvent and plant material used. Ardathoda vasica leaf extract gave the highest inhibition in all five solvents; methanol, Chloroform, Petroleum ether, Ethanol, and Sterilized distilled water have shown the maximum zone of inhibition of all three fungal species. The second highest inhibition was observed in all Azadirachta indica extracted in solvents; Methanol, Chloroform, Petroleum ether, ethanol, and Sterilized Distilled Water with the higher inhibition zones of all three fungal species. Among the solvents, ethanol performed the best, having the highest inhibition zone in between 10 - 14 mm range including in the control with solvent only followed by moderate performances in the other plant extracted solvents; Methanol and Chloroform, while distilled water and petroleum ether were least effective against all fungal species. In the present study, the natural product extracts identified to inhibit fungus species with high potency are leading candidates for antifungal identification. The most effective plant extracts against the Rhizopus stolonifer are Ardathoda vasica and Azadirachta indica. The study revealed a promising prospect for the utilization of selected plant extracts in postharvest diseases control and the potential to develop bio fungicides using botanicals.*

Keywords: *antifungal effects, plant extracts, solvents, zone of inhibition*

1.0 INTRODUCTION

Post-harvest losses of fruits and vegetables mainly occur due to improper postharvest practices, diseases, and lack of facilities and technology to extend their storage life. Among all the factors for reducing the losses on food supply, postharvest diseases are a major factor that causes the losses by limiting the duration of storage (Karabulut and Baykal, 2004; Liu et al, 2005). It is estimated that post-harvest diseases destroy 10-30 % of the total yield of crops and in some perishable crops, especially in developing countries; they destroy more than 30% of the crop yield (Agrios, 2005). Several species of fungi are responsible for post-harvest deterioration. They belong to different genera including *Penicillium, Botrytis, Alternaria, Gleosporium, Mucor, Rhizopus, Fusarium, Monilinia* and *Aspergillus (*Liu et al, 2013). Generally, controlling these pathogens is quite efficiently

performed by synthetic chemical fungicides. However, it is recorded the increased resistance against the limited number of authorized chemical fungicides at present has increased the efforts of finding alternative or complementary control measures among the researchers (Ippolito et al., 2005; Smilanick et al., 2008; Droby et al., 2009; Sanzani et al., 2009; Sharma et al., 2009; Mari et al., 2010).

Extracts containing different classes of phenolic compounds from many plants have recently gained popularity as well as scientific interest for their antibacterial and antifungal activity (Lee et al., 2007; Verástegui et al., 2008; Santas et al., 2010, Rauha et al., 2000; Al-Zoreky, 2009). Phenolic compounds represent a rich source of biocides and preservatives that have been explored for a long time as postharvest alternative control means (Lattanzio, 2003). The components with phenolic structures, like carvacrol, eugenol, and thymol were highly active against the plant pathogens. With its rich biodiversity, Sri Lanka is blessed with many unexplored wild herbaceous species with different capacities and which are possible to incorporate into crop development by means of fertilizers or as pesticides. They are interesting from an ethnobotanical point of view since a lot of them are used in Sri Lanka as a source of drugs in traditional and Ayurveda medicine. In fact, they are known as a rich source of antioxidant, anti-inflammatory, diuretic, antibacterial, and antiviral active substances, with medicinal as well as cosmetic applications (Yukawa et al.,1996; Dhiman and Chawla, 2005; Wang et al., 2006; DiVenere et al., 2009). The literature claims a very low number of explorations of antimicrobial activity of phenolic obtained from wild species against postharvest fungal pathogens. Therefore, the objective of the present study was to evaluate the *in vitro* antifungal activity of different solvent extracts of five medicinal plants. Preliminary data were analyzed to study the efficacy of the different solvent extracts of selected plants in preventing the growth of three post-harvest fungal species.

2.0 METHODOLOGY

2.1 Plant Materials

Plants of five tropical plant species (*Pistia stratiotes, Adhathoda vasica, Ricinus communis, Clerodendrumin fortunatum, Azadirachta indica*) were collected from Low Country Wet Zone in Sri Lanka and classified according to botanical name and family (Table 1). Plant parts were collected and transported to the laboratory where they were cleaned washed with distilled water followed by washing with 5% of Sodium hypochlorite (NaOCl) and added with a few drops of Tween-20.

Table 1: Plant - extracts

Scientific name	Common name	Plant Part used
Pistia stratiotes	Water lettuce	Leaves
Adhathoda vasica	Adathoda	Leaves
Ricinus communis	Castor plant	Seeds
Clerodendruminfortunatum	Hill glory bower	Leaves
Azadirachta indica	Margosa	Seeds

2.2 Preparation of plant Crude Extracts

Analytical grade solvents; methanol, petroleum ether, ethanol, chloroform, and Sterilized distilled water were used as extraction solvents. Plant tissues were homogenized by following the method described by Gurjar et al., in 2012 with slight modifications. Plant materials were ground by using sterile mortar and pestle by adding sample: solvent as 1:10, subjected to shaking at 100 rpm for 24 hours at room temperature. Extracts were subjected to filtration (What-man 42 filter paper), where three-time filtration was done with the respective solvent each time followed by centrifugation at 4000 rpm for 20 minutes. The filtrate was concentrated through a rotary evaporator until a sticky dark green crude extract was obtained at 700 ppm pressure and 50°C for Methanol, Ethanol, Petroleum Ether, and Chloroform and at 0°C for distilled water. The crude extracts were kept in an airtight container and stored at 4◦C until further use.

2.3 Preparation of the fungal cultures

Rhizopus stolonifer, Colletotrichum gloeosporioides, and *Aspergillus spp* isolates were collected from the Department of Botany, Faculty of Natural Science, The Open University of Sri Lanka, Nawala, Nugegoda. The fungal cultures were subcultured from potato dextrose agar (PDA) slants into the plates of freshly prepared potato dextrose agar growth medium.

2.4 Antifungal assay

Antifungal activities were performed through the Agar Disc Diffusion method (Bauer et al, 1966; Rios et al, 1988; Alzoreky et al, 2003) and recommended by the NCCLS (National Committee for Clinical Laboratory Standards). The 100 mg/ml plant extract stock solutions were prepared by dissolving in relevant solvent for trials. Each prepared was filtered using sterilized SF13-N-22P Nylon welded syringe filter (ALWSci technologies. www.chinasepta.com) with 0.22 µm size pores.100 µg/ml concentration test solutions were prepared by adding each solvent as per the equation, $C_1V_1=C_2V_2$ where C_1 and V1 are the concentration and volume of stock solution and C_2V_2 are the concentration and volume of test solution respectively. Organisms were subcultured on Potato Dextrose Agar (PDA) at 30°C for 10 days. Conidia were harvested in sterile saline, and using a hemocytometer, the conidial suspension was adjusted to 1.0×10^6 conidia/ml. Mueller-Hinton (MH) agar plates were streaked evenly with a sterilized swab dipped into the standardized inoculums suspension. Crude extract impregnated discs were aseptically transferred on the inoculated agar plates and left to be incubated at 28 °C for 72 hrs to 7 days (Salie et al., 1996; Baris et al., 2006). The clear zones of inhibition around the test crude extract disc were measured for any indication of antimicrobial activity. Solvents were used as negative controls. All assays were carried out in triplicate.

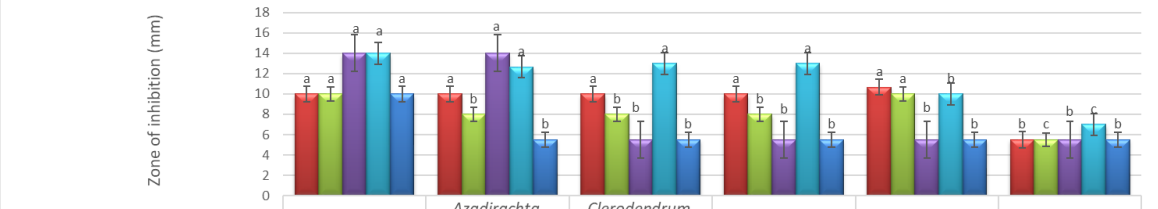
2.5 Data and statistical analysis

Microsoft Excel software (version 13) was used for basic descriptive statistical analysis. Linear growths (LG) for antifungal activities were calculated by measuring the inhibition zones' diameter in millimeters. Antifungal activities were measured by the formula described by Mahmood et al., 2012. Data were analyzed by one-way ANOVA with 95% level of confidence ($P < 0.05$).

3.0 RESULTS AND DISCUSSION

Rhizopus stolonifer, Colletotrichum gloeosporioides and *Aspergillus* sp. were very sensitive to *Adhathoda vasica* - leaves, *Azadirachta indica*- seeds, *Ricinus communis* - seeds, *Clerodendrum*

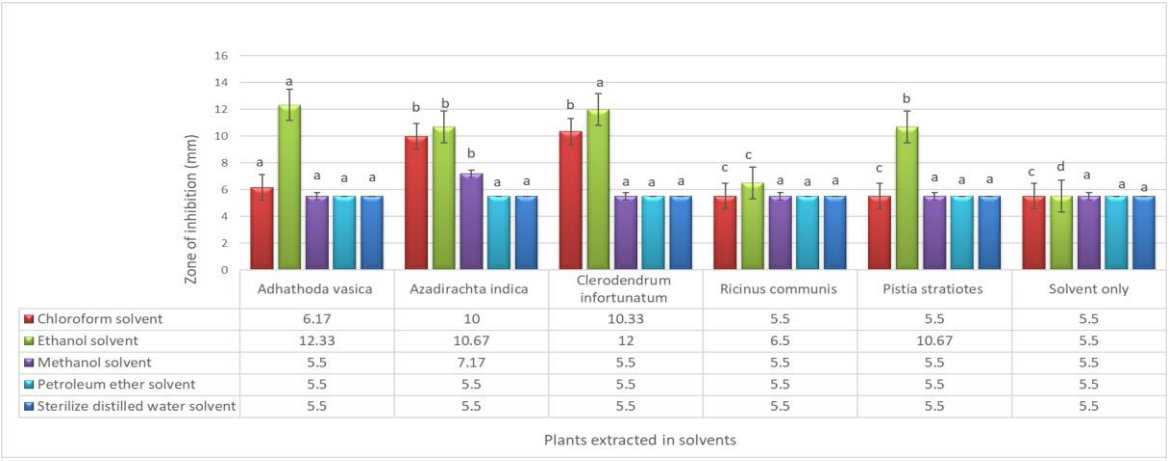
infortunatum-leaves and *Pistia stratiotes*- leaves as colony growth of this fungus was inhibited or reduced when the growth media was amended with plant extracts. The result of the *in vitro* screening tested against *Rhizopus stolonifer*, *Colletotrichum gloeosporioides,* and *Aspergillus* spp. revealed that there was a significant difference (*p<0.005) in antifungal effect among treatments when using different solvents; methanol, ethanol, chloroform, petroleum ether, and sterilized distilled water. There was a significant difference among the antifungal effect of five plant extracts; Adhathoda *vasica*, *Azadirachta indica*, *Ricinus communis*, *Clerodendrum infortunatum* and *Pistia stratiote* leaves in five different solvents; methanol, ethanol, chloroform, petroleum ether, and sterilized distilled water in inhibiting the colony growth of *Rhizopus stolonifer, Colletotrichum gloeosporioides,* and *Aspergillus* spp. Figure 1, 2, and 3 indicates the selected fungal colony inhibition in the present study. According to figure 01, *Adhathoda vasica* had the highest zone of colony inhibition (14 mm) among plant extracts in petroleum ether and Ethanol solvents. *Azadirachta indica* were also capable of inhibiting radial colony growth (14 mm, 12.67 mm) of the fungus *Rhizopus stolonifer.* Further, figure 01 indicates the *Ricinus communis* and *Clerodendrum infortunatum* ethanolic extracts strongly inhibited (13 mm) the mycelial growth of *Rhizopus stolonifer*. The inhibition of the mycelium growth of the *Rhizopus stolonifera* was visible in the negative control with solvents only having <8 mm inhibition. This could be due to chemical inhibition of fungi. However, when compared with other plant extracts *Adhathoda vasica and A. indica* had significantly higher mycelium inhibition in *Rhizopus stolonifer*. The previous studies revealed that these inhibitory activities are due to the direct toxic effects of plant extracts on the pathogens (Bhutia et al.,2015; Chowdhury et al., 2017). Mohamed and EI-Hadidy in 2008, detected those antifungal activities of the plant extracts also is a course of the presence of secondary plant metabolites such as terpenoids, phenols, flavonoids, alkaloids. Further, Tunwari and Nahunnaro revealed in 2014 that the presence of these plant metabolites indicates the fungicidal properties of natural plant products and their potential to control plant diseases. As per the Tijjani et al. (2014) and Chowdhury et al. (2017) the increase in the concentration of plant extracts implied an increase in the active ingredients of the crude extracts which act on the test pathogens thereby affecting its physiological processes, lowering the growth of the pathogens.



	Adhathoda vasica	Azadirachta indica	Clerodendrum infortunatum	Ricinus communis	Pistia stratiotes	Solvent Only
Methanol solvent	10	10	10	10	10.67	5.5
Chloroform solvent	10	8	8	8	10	5.5
Petrolium ether solvent	14	14.0	5.5	5.5	5.5	5.5
Ethanol solvent	14	12.67	13	13	10	7
Sterilized Distilled water solvent	10	5.5	5.5	5.5	5.5	5.5

Fig. 01: Total Effect of leaf and seed extracted in different solvents; Chloroform, Ethanol, Methanol, Petroleum ether and Sterilized Distilled water, on mycelial growth of Rhizopus stolonifera

In this study, the five plants extract with five solvents screened showed different effects on C. *gloeosporioides*. This study has demonstrated the possibility of using extracts from some plants to control the mycelial growth of *C. gloeosporiodes*. Ethanol extract of *Adhathoda vasica* showed (12.33 mm) the highest inhibition of mycelium of *Colletotrichum gloeosporioides*. *Clerodendrum infortunatum* and *Azadirachta india* also inhibited (12 mm, 10.67 mm) colony inhibition of the fungus. Ethanolic *Ricinus commuis* extract gave 6.5 mm inhibition among other solvents. Only ethanol solvent had a slightly high zone of inhibition (figure 02). According to figure 02, *A. indica* showed higher inhibition towards the *C. gloeosporiodes* than other plant extracts.

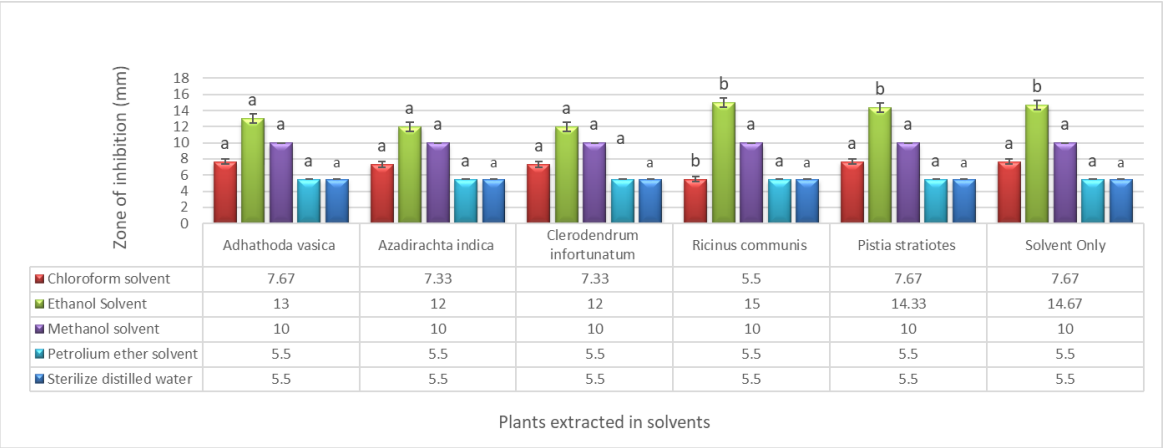


	Adhathoda vasica	Azadirachta indica	Clerodendrum infortunatum	Ricinus communis	Pistia stratiotes	Solvent only
Chloroform solvent	6.17	10	10.33	5.5	5.5	5.5
Ethanol solvent	12.33	10.67	12	6.5	10.67	5.5
Methanol solvent	5.5	7.17	5.5	5.5	5.5	5.5
Petroleum ether solvent	5.5	5.5	5.5	5.5	5.5	5.5
Sterilize distilled water solvent	5.5	5.5	5.5	5.5	5.5	5.5

Fig 02: Total Effect of leaf and seed extracted in different solvents; Chloroform, Ethanol, Methanol, Petroleum ether, and Sterilized Distilled water, on mycelial growth of *Colletotrichum gloeosporioides*

Ethanolic *Ricinus communis* seeds extraction gave 15 mm inhibition of *Aspergillus* sp. as well as ethanolic *Adhathoda vasica* leaves *Azadirachta indica seeds, Clerodendrum infortunatum leaves,* and *Pistia stratiotes*- leaves extracts were capable of 13 mm,12 mm, 12 mm, 14.33 mm inhibition of mycelium growth of *Aspergillus* sp. Only consider about solvent Ethanol also gave the highest inhibition (14.67 mm) than the other four solvents. This has to be further studied to clarify the effects of ethanol on growth inhibition on *Aspergillus* sp. In addition, chloroform, as well as methanol showed higher inhibition in control, were required further studies or clarifications. Moreover, none of the extracts of *Adhathoda vasica,* as well as *Azadirachta indica* showed significant inhibition on *Aspergillus* sp.

Adhathoda vasica and *Azadirachta indica* extracted in ethanol showed significantly higher growth inhibition of all three fungal species, *R. stolonifer C. gloeosporioides* and *Aspergillus* sp. beyond that, *Ardathoda vasica and Azadirachta indica* gave higher mycelium growth inhibition on *Rhizopus stolonifer.* Asdaq and Inamdar in 2010 reported that the aqueous extracts of plants generally showed antimicrobial activities. As per the findings of Belewa et al, in 2011, extract of *A. indica* and *Chromolalenaodonata* inhibited the growth of *A. niger*, *F. oxysporum, R. stolonifer* and *Geotrichum candidum*. Anukworji et al, 2012 stated that the ability of the leaf extract of *A. indica, R. comunis*, and *M. indica* to inhibit growth and spore germination of *R. stolonifer* and *F. oxysporum* could be due to the presence of fungi toxic compounds in the extracts of the three plant species. In the present study, *A. indica* showed high effectiveness in Aspergillus sp. Like *Aspergillus niger* and *R. stolonifer.* Among the five solvents, moderate antifungal activity was shown by plants, *Ricinus communis,* and *Clerodendrum infortunatum* extracted in all solvents other than ethanol extracted.



	Adhathoda vasica	Azadirachta indica	Clerodendrum infortunatum	Ricinus communis	Pistia stratiotes	Solvent Only
Chloroform solvent	7.67	7.33	7.33	5.5	7.67	7.67
Ethanol Solvent	13	12	12	15	14.33	14.67
Methanol solvent	10	10	10	10	10	10
Petrolium ether solvent	5.5	5.5	5.5	5.5	5.5	5.5
Sterilize distilled water	5.5	5.5	5.5	5.5	5.5	5.5

Fig 03: Total Effect of leaf and seed extracted in different solvents; Chloroform, Ethanol, Methanol, Petroleum ether, and Sterilized Distilled water, on mycelial growth of *Aspergillus* spp

Further, as per figures 02 and 03, ethanol had the highest fungal inhibition, with respect to the solvents used for extraction in the present study. Figure 02, explain the higher inhibition zone in between 10 - 14 mm range including the control with solvent only having the inhibition zone of 7 mm followed by other extracts; Methanol and Chloroform, while distilled water and petroleum ether were least effective against *R. stolonifer*. This is an indication that the active principles in these mosses are predominantly polar. These results are in agreement with those of Alam et al. (2011) as well as the results of Femi-Adepoju et al, (2014) that aqueous extract of the liverwort *Dumortiera hirsute* was found to inhibit a number of phytopathogenic fungi mediated by different modes of action such as spore germination inhibition, development of anomalies in the hyphae, formation of the flaccid cell wall and granulated cytoplasm. The present results also partially agree with those of Basile et al. (1998) in which acetone extract of *Lunularia cruciate* (a bryophyte) showed no activity against *Candida albicans* and *Aspergillus niger* as well as the findings of Amadioha (2001) who investigate the effects of *Cymbopogon citratus*, *Azadirachta indica* (Neem) and *Ocimum gratissimum* extracts on controlling of the growth of *Rhizopus oryzae in vitro* and *in vivo*. In the tests involving acetone and petroleum ether as solvents, the absence of antifungal activity is suspected to be due to the presence of non-polar molecule(s) in the extracts resulting in the inability of the molecules to cross the fungal cell wall (Basile et al., 1998). Therefore, in the present study the highest mycelium inhibition was achieved by the *Ardathoda vasica* and *Azadirachta indica* plant extracts against the *Rhizopus stolonifer*.

4.0 CONCLUSION

In the present study, the natural product extracts identified to inhibit *Rhizopus stolonifer, Colletotrichum gloeosporioides* and *Aspergillus* sp with high potency are leading candidates for antifungal identification. The most effective plant extracts against *all three fungal species* are *Ardathoda vasica* leaves and *Azadirachta indica seeds*. The present study also confirms the in-vitro synergistic effect of *Ardathoda vasica* leaves and *Azadirachta indica against the Rhizopus stolonifer. In-vitro* experiment showed that ethanolic *Ardathoda vasica* leaves and *Azadirachta indica seeds* resulted in highest colony inhibition on *Rhizopus stolonifer*. Isolation of the active compounds from ethanolic extracts could lead to improved antifungals for use in agriculture to preserve food crops as well as in the pharmaceutical industry for the treatment of mycoses. Moreover, the results of the study will form the base for the selection of plant species for further investigation in the potential discovery of new natural bioactive

compounds. Further studies that aim at the isolation of antibacterial active constituents from the plant have to be initiated while estimating the Minimum Inhibitory Concentration (MIC) of the plant extracts in different solvents.

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Evaluation of the Use of Recycled Construction and Demolition Waste for Road Construction in Sri Lanka

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Abstract - *The annual amount of construction and demolition waste produced in Sri Lanka is about 4.0 million tons and this has already become an environmental problem. In order to provide a sustainable environment, alternative solutions of utilizing these waste materials are needed. On the other hand, highways are constructed rapidly, causing depletion of natural resources like gravel. Therefore, this paper investigates the feasibility of using recycled construction and demolition waste in the road construction of Sri Lanka for road bases and sub-bases to provide a feasible solution for the said problem. This study used Recycled Concrete Aggregates and Crushed Brick and gravel from an excavation site where gravel was the control sample. Different mixes with various proportions of these materials were prepared and subjected to a series of tests in accordance with ICTAD specifications of Sri Lanka to determine their physical and mechanical properties. The results of particle size distribution, consistency tests, compaction test and CBR test indicate that the potential of using these materials in embankments, lower sub base, upper sub base for flexible pavement and road shoulder material. Moreover, the geotechnical properties of cement-treated C&D materials were also evaluated and found to be satisfactory for road and sub-base.*

Keywords: *Cement treated aggregates, Crushed Bricks, Demolished Concrete, flexible pavement, sub base*

Nomenclature

CB – Crushed Bricks
C_C - Coefficient of Gradation
CBR – California Bearing Ratio
CD – Construction and Demolition
C_U – Uniformity Coefficient
ICTAD – Institute of Construction Training and Development
MDD – Maximum Dry Density
OMC – Ordinary Moisture Content
OPC – Ordinary Portland Cement
RCA – Recycled Concrete Aggregates
UCS – Unconfined compressive strength
USCS - Unified Soil Classification System

1. INTRODUCTION

Research on the use of commercial and industrial waste materials in civil engineering applications has generated interest in recent years. The reuse of these recycled materials will result in a low carbon footprint, considering that these recycled materials have significant

carbon savings compared with extracting virgin quarried materials. Construction and demolition (C&D) materials constitute a major proportion of waste materials present in landfills worldwide. The C&D materials have been used in recent years in various civil engineering applications such as roads, embankments, pipe bedding, and backfilling.

Economic, industrial and population growth in Sri Lanka will generate increasing amounts of waste materials that must be disposed of. As the volume of wastes continues to grow, the approval and availability of facilities for waste processing and proper disposal will become more difficult to obtain. Out of this waste the major proportion result in C&D waste; i.e. mainly demolished concrete and crushed bricks. These materials are stockpiled annually.

Evidences show that approximately 40% of waste generated globally originates from construction and demolition of buildings (Roach, 2001). C&D waste constitutes an increasingly significant problem in society leading to harmful effects environmentally as well as economically, not so much because of its hazardous nature, as it can be inert, but because of the volume generated which renders sustainable management and disposal problematic. According to Deiyagala (2017), the annual amount of construction and demolished waste produced in Sri Lanka is about 4.0 million tons and the management of that waste has already become an environmental problem. Therefore, there is urgency on finding innovative ways of recycling and reusing these materials.

In some countries, over 50% of C&D waste is sent to land filling areas. However, C&D waste is possible to be developed, that can be used in road constructions, i.e. in embankment, sub bases etc. Relevant tests have been carried out in developed countries and laboratory test results have shown positive results that the materials are viable to be used in road construction (Mohammadina *et al.* 2015).

Currently, the Road Development Authority in Sri Lanka uses only traditional materials for these purposes as per the Sri Lankan specifications (ICTAD, 2009) i.e. for road constructions, materials taken from gravel excavations are used, but these resources are depleting due to large scale excavations and rapid development in road constructions. According to Taha, et al. (2014) waste materials are commonly used in construction projects in order to save natural resources for future generations. Road construction is one of the main users of these natural resources. Utilizing these materials in unbound base/sub-base construction will provide sustainable development in a country by saving virgin materials, conserving energy and diverting materials from landfills Therefore, this research will assess the suitability of recycled concrete aggregates and brick blends as embankment, road sub base and shoulder materials and gravel surfacing, and the improvement of the strength parameter of cement treated blended samples. This would provide substantial benefits to the industry in terms of reduced material supply and waste disposal cost, increased sustainability and reduced environmental impact.

Thus, the main objective of this research study was to investigate the potential for constructing road bases and sub-bases from waste materials generated in Sri Lanka. To meet this objective, physical and mechanical properties of C&D, crushed bricks were determined. Then combinations of C&D, CB and gravel (control sample) were subjected to Atterberg Limit tests, compaction and California Bearing Ratio (CBR) tests. Results were compared with ICTAD Specifications (2009) to establish the viability of using such materials in road base and sub-base structure, shoulder material and embankments.

2. PREVIOUS RESEARCH

Although the use of demolished construction waste is not still popular in Sri Lanka, internationally, there has already been much research done and applications identified in this regard.

Arulrajah (2012) reports of a laboratory investigation of the geotechnical properties of RCA. The properties of RCA were compared with state road authority requirements to assess its performance as a pavement sub-base material. The experimental programme consisted of tests such as particle size distribution, modified Proctor compaction, particle density, water absorption, CBR, Los Angeles abrasion, pH, organic content, static triaxial, and repeated load triaxial tests. The Los Angeles abrasion loss tests indicated that the RCA is durable. CBR values were found to satisfy the local state road authority requirements for a lower sub-base material. Repeated load triaxial tests established that the RCA would perform satisfactorily as a pavement sub-base material in the field.

Jayakody et al. (2012) investigated performance characteristics of blends of reclaimed asphalt pavement (RAP) with RCA. A series of "repeated load tri-axial (RLT)" test was conducted on RAP blended RCA samples to evaluate the elastic and plastic deformation characteristics with increase of load cycles. The elastic deformation was characterised by resilient modulus and slightly dropped with increase of RAP from 0, 5, 10 to 15% in RCA. Moreover, they have observed a trend of small increase of the plastic deformation of the RCA with the increase of rap portion. However, presence of RAP up to about 15% in RCA did not significantly affect on the accumulation of permanent strain.

An extensive laboratory program is conducted to study the feasibility of using RCA mixed with traditional limestone aggregate (LSA) by Behiry (2013). Moreover, the influence of mixture variables on the mechanical properties of cement treated recycled aggregate (CTRA) was also investigated. The results show that the adding of RCA improves the mechanical properties of the mixture where the UCS is taken as an important quality indicator.

Mohommadinia et al., (2015) investigated cement treated reclaimed asphalt pavement (RAP), RCA, and CB to assess their performance in pavement base/sub base applications. The effect of curing duration on the strength of the C&D materials was analyzed by conducting unconfined compression strength and repeated load triaxial tests. The RAP required 2% cement (by weight) and either 7 or 28 days of curing to meet the local road-authority requirements, whereas RCA and CB required 4% cement and 28 days of curing. It was reported that the RAP exhibited the highest strength in all cases, with the same cement content and for the same curing duration, followed by RCA and CB. The resilient moduli of C&D materials increased with an increase in cement content, curing duration, and confining pressure. Humidity curing was found to play an important role in the strength development of cement-treated C&D materials. This study indicates the potential of using cement-treated C&D materials for pavement base/sub base applications.

A series of extensive geotechnical laboratory tests was undertaken on CB blended with gravel in the varying proportions of 100%, 50%, 30% and 15%. Particle size distribution tests, Atterberg limit tests, Modified Proctor compaction tests and 4-day soaked CBR at 98% MDD (Modified) tests were carried out. The geotechnical properties obtained by the tests were compared with ICTAD requirements of sub-base specifications for pavement base and sub-base applications. The grading of all the blends tested satisfied the grading requirement for sub-base construction as per the ICTAD specifications. The 100% recycled brick sample achieved a maximum dry density of 2020 kg/m3 and a CBR value of 113% and satisfied the standard requirement. In addition, the blend of 50% crushed bricks and 50% gravel also satisfied the Atterberg limit dry density (1779 kg/m3) and CBR value (32%) requirements of ICTAD standards. The findings reveal that the two blends of recycled bricks and gravel are viable materials for pavement base or sub-base as a substitute material for gravels (Wijewardena, 2015).

Sirin (2013) reports of the evaluation of reclaimed asphalt pavement (RAP) aggregates and excavation waste (EW) materials obtained from road and building construction projects in for road bases and sub bases. Different combinations of such materials were prepared and subjected to a series of tests in accordance with Qatar's Construction Specifications (QCS) to determine their physical and mechanical properties. Results indicate a weak potential for

using RAP aggregates, EW materials, or a combination of the two in road bases and sub-bases.

Pourkhorshidi, (2020) reports the use of the construction and demolition waste aggregates in unbound layers of pavements and compare the in-hand results from various engineering assessments of these aggregates and mixes. A number of tests and evaluations are applied in order to enhance the required quality and durability of the pavements under given traffic volumes traffic loads and climate actions. Although the unbound recycled aggregates (RA) are mainly used in the lower layers, such as sub grade, capping, sub-base and base, he but suggested that the material can be used in rural roads for bound layers, towards the surface of the structure and may be for constituents of bound layers and of novel surfacing applications.

3. MATERIALS AND METHODOLOGY

Lateritic soil, crushed bricks and demolished concrete were the main materials used. Different blends of these materials were prepared and subjected to a series of tests in accordance with ICTAD specifications of Sri Lanka to determine their physical and mechanical properties.

A laboratory evaluation was also carried out to determine the engineering properties of cement-treated C&D materials. To assess this all the blends were treated with 5%, 6% and 7% of Ordinary Portland Cement (OPC) to evaluate the adequacy of strength and to assess their performance in pavement base/sub base applications.

All the results were compared with ICTAD Specifications (2009) to establish the viability of using such materials in the base and sub-base structures, shoulder material and road embankments.

3.1 Soil

Soil was collected from a borrow pit at Galagedara, Kandy which is used by Road Development Authority (RDA), Sri Lanka for road construction. This was in conformity with RDA material classifications. The soil from this borrow pit was classified as "Type I" soil.

3.2 Demolished Concrete

Demolished Concrete was from the Construction Waste Management Center, Galle.

3.3 Crushed Bricks

The cement containing particles attached to bricks were removed and were hand crushed to obtain the particle sizes specified in ICTAD specifications for road constructions.

3.4 Tests

The samples (soil, demolished concrete and crushed bricks) brought to the laboratory were tested individually to determine the material properties prior to mixing according to standard test methods. Following tests were conducted. The particle size distribution of samples was analyzed According to BS 1377-2:1990 (BS 1377: Part 2, 1990). Specific gravity of materials was obtained using pycnometer method as described in BS 1377: Part 2, 1990 for determination of particle density. Water absorption of the soil sample was determined using the oven drying method as described in BS 1377-2:1990.

To obtain the liquid limit and plastic limits of materials, the Casagrande Apparatus method was used as per the BS 1377-2:1990 part 2.

Both Standard Proctor Compaction test (BS 1377-2:1990) and Modified Proctor Compaction test (AASHTO T 180) was performed to determine the MDD and OMC of the samples.

For CBR test, samples were compacted at 95% and 98% MDD were soaked for 96 hours and the CBR values were evaluated as per the test procedure AASHTO 193.

Unconfined compressive strength test on cement treated materials were conducted as per the ASTM D2166. The cement treated samples were compacted according to AASHTO T180, heavy compaction using 4.5kg hammer with a free fall of 450cm. In order to achieve a 97% MDD compaction, samples were compacted in into 5 layers, giving 25 blows to each layer at its OMC. A cylindrical mould having an average height of 116.2 mm and an average diameter of 101.5 mm was used for the compaction. Compacted samples were extruded carefully and care was taken not to damage the surface area, especially top and bottom surfaces and left in humid conditions for 24 hours. Then the samples were cured in water for 7 days prior to test for its unconfined compressive strength using a standard compressive strength testing machine.

3.5 Material mix proportions

Different combinations of samples including the control sample were prepared as described in Table 1 and tests were conducted to evaluate the necessary engineering properties of each mixes and were compared with the specifications used by Road Development (ICTAD, 2009) of Sri Lanka to assess the suitability in road construction.

Table 1: Material mix proportions in sampling.

Test Series	Sample Code	Material Percentage (%)		
		Natural Gravel	Crushed Concrete	Crushed Bricks
	CS	100	-	-
T1	T1S1	65	35	-
	T1S2	60	40	-
	T1S3	55	45	-
	T1S4	50	50	-
T2	T2S1	65	-	35
	T2S2	60	-	40
	T2S3	55	-	45
	T2S4	50	-	50
T3	T3S1	65	17.5	17.5
	T3S2	60	20	20
	T3S3	55	22.5	22.5
	T3S4	50	25	25

3.6 Material Mixing

In order to maintain the consistency of the mix, first coarse and fine particles of each RCA and CB was mixed for several minutes separately and after it was mixed with soil in different proportions until a homogeneous mix was formed using a mechanical mixer.

4. RESULTS AND DISCUSSION

A summary of test results of physical and geotechnical properties of gravel, RCA and CB are presented in Table 2 and 3 respectively.

Table 2: Physical properties of gravel, RCA, CB.

Physical Property		Natural Gravel	RCA	CB
Standard test method	**Property**			
Sieve Analysis Test (BS 1377)	Fine Percentage (%)	<5%	<5%	<5%
	Cu	8.00	17.00	25.44
	Cc	1.62	1.75	2.44
	USCS Classification	SW-SM	SW-SM	SW-SM
Pycnometer Test (BS 1377)	Specific Gravity	2.80	2.80	2.67

Table 3: Geotechnical properties of gravel, RCA, CB.

Geotechnical Property		Natural Gravel	RCA	CB
Standard test method	**Property**			
Consistency Test (BS 1377)	Liquid Limit (%)	33	-	-
	Plastic Limit (%)	24	-	-
	Plasticity Index (%)	9	N.O.	N.O.
Standard Proctor Compaction (BS 1377)	Maximum Dry Density (kg/m^3)	2100	1910	1560
	Optimum Moisture Content (%)	11.2	15.2	23.8

4.1 Particle Size Distribution

Figures 1, 2, 3 and 4 illustrate the Sieve Analysis Test results of virgin materials and the blends. From these it can be seen that gradation curves of the virgin materials as well as the blends lie within the specified limits of ICTAD (2009) standards.

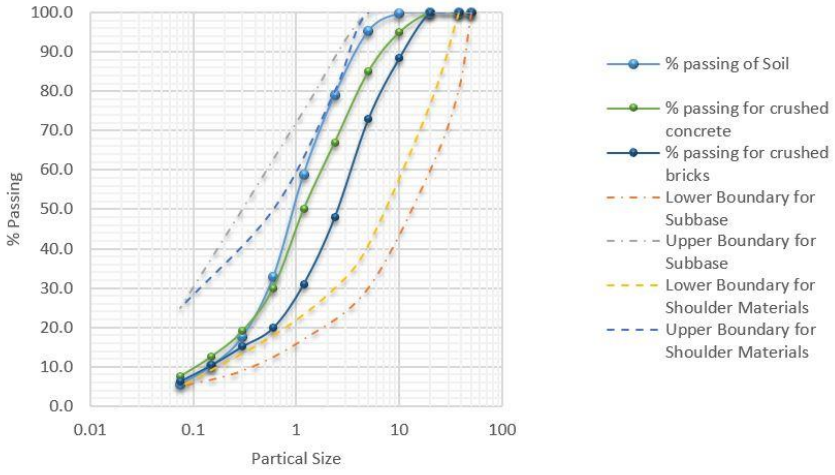

Figure 1: Sieve Analysis Test Results of Gravel, RCA and CB.

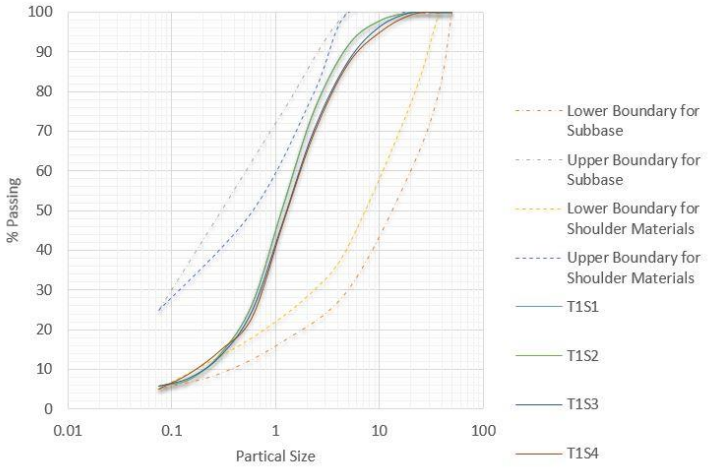

Figure 2: Sieve Analysis Test Results of Gravel + RCA Series.

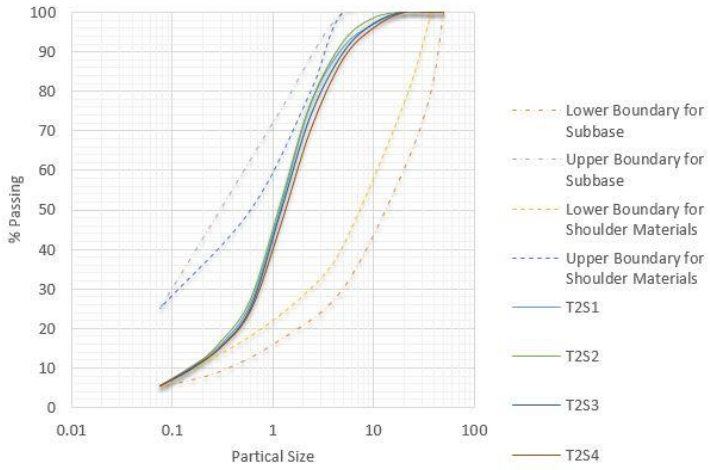

Figure 3: Sieve Analysis Test Results of Gravel + CB Series.

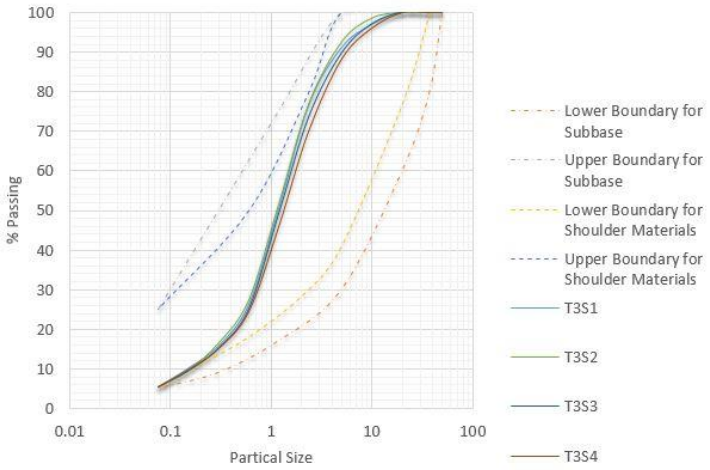

Figure 4: Sieve Analysis Test Results of Gravel + RCA + CB Series.

4.2 Atterberg Limits

The Liquid Limit, Plastic Limit and the Plasticity Index of the gravel sample were 33.2%, 23.6% and 9.6% respectively. The virgin RCA and CB were found to be non-plastic.

Table 3 illustrates the consistency limits of blends. Accordingly, a gradual decrease in Liquid Limit, an increase in Plastic Limit and a decrease in Plasticity Index in comparison to gravel was observable with the increase of replacement ratio of RCA and CB. This loss of consistency of natural gravel may be due to the fact that RCA and CB being non plastic.

Table 3: Consistency Limit test results

Material Percentage	Liquid Limit (%)	Plastic Limit (%)	Plasticity Index (%)
100% gravel	33.2	23.6	9.6
65 % gravel + 35% RCA	29.9	23.6	6.3
60% gravel + 40% RCA	29	24.1	4.9
55% gravel + 45% RCA	26.9	24.7	2.4
50% gravel + 50% RCA	26.6	25.9	0.7
65% gravel + 35% CB	29.3	23	6.3
60% gravel + 40% CB	28.4	23.9	4.5
55% gravel + 45% CB	26.7	25.1	1.6
50% gravel + 50% CB	25.9	25.3	0.6
65% gravel + 17.5% RCA + 17.5% CB	29.9	23.7	6.2
60% gravel + 20% RCA + 20% CB	29.6	24.7	4.9
55% gravel + 22.5% RCA + 22.5% CB	27.8	25.1	2.7
50% gravel + 25% RCA + 25% CB	26.3	25.6	0.7

The Table 4 presents the Liquid Limits of the blends along with the respective ICTAD specifications. As can be seen from Table 4 except for the ICTAD (2009) requirement of sub bases of rigid pavement rest are complied with the specifications. The results of all the blends were found to exceed the maximum specified value of 25%.

Table 4: Liquid Limit values and the respective ICTAD (2009) standards.

Soil Percentage (%)	Liquid Limit for Each Blend (%)			Specifications					
	Gravel + RCA	Gravel + CB	Gravel + RCA + CB	Upper Limit for Embankment Type I	Upper Limit for Embankment Type II	Upper Limit for Upper Sub base (Flexible)	Upper Limit for Upper Sub base (Rigid)	Upper Limit for Lower Sub base	Upper Limit for Earthen Road Shoulders
100	33.2	33.2	33.2	50	55	40	25	40	55
65	29.9	29.1	29.9	50	55	40	25	40	55
60	29	28.5	29	50	55	40	25	40	55
5	27.2	27	27.2	50	55	40	25	40	55
50	26.9	25.9	26.9	50	55	40	25	40	55

4.3 Maximum Dry Density

Table 5 illustrates the results of the Modified Proctor compaction test.

The maximum dry density of gravel is is found to be higher than that of RCA and CB. As can be seen the maximum dry densities have decreased with the increase of C&D waste percentage and OMC vice versa. However, as per the figure 5, 6, 7 it can be concluded that all the blends tested did comply with the ICTAD (2009) specification for all the structures of road construction

Table 5: Standard Proctor Compaction Test Results.

Test Sample	OMC (%)	MDD (kg/m³)
100% gravel	9	2170
65 % gravel + 35% RCA	8.4	2155
60% gravel + 40% RCA	9.1	2145
55% gravel + 45% RCA	9.2	2095
50% gravel + 50% RCA	9.2	2075
65% gravel + 35% CB	9.5	2100
60% gravel + 40% CB	8.9	2060
55% gravel + 45% CB	9.8	2070
50% gravel + 50% CB	10.5	2000
65% gravel + 17.5% RCA + 17.5% CB	8.5	2100
60% gravel + 20% RCA + 20% CB	9	2080
55% gravel + 22.5% RCA + 22.5% CB	8.9	2030
50% gravel + 25% RCA + 25% CB	10.1	2010

Figure 5: Comparison of MDD for gravel + RCA samples with standards

Figure 6: Comparison of MDD for gravel + CB samples with standards.

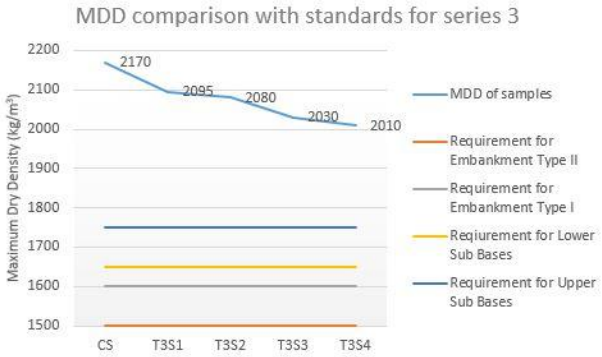

Figure 7: Comparison of MDD for gravel + RCA + CB samples with standards.

4.4 California Bearing Ratio

A summary of CBR Test results are presented in Figures 8 and 9. As the percentage of C&D is increased in the mixture there was an increase in the CBR value. Gravel + CB blends showed a significant increase in the CBR values than the Gravel + RCA blends. The CBR at 98% MDD for natural gravel was only 28% which was below the specified value for upper sub bases i.e., 30%. However, with increasing the proportion of C&D waste the CBR value can be increased.

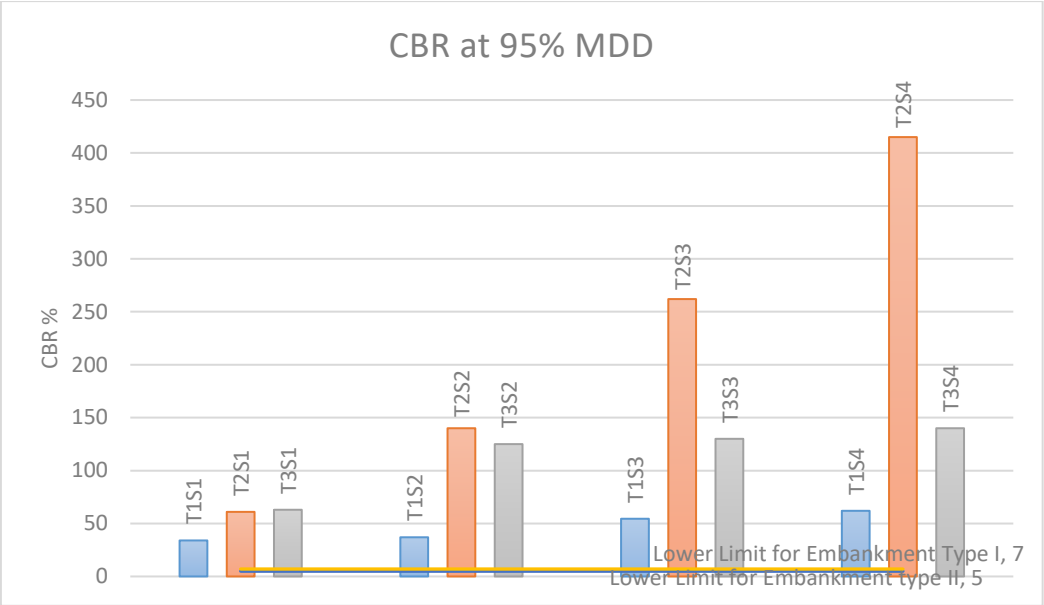

Figure 8: CBR at 95% MDD

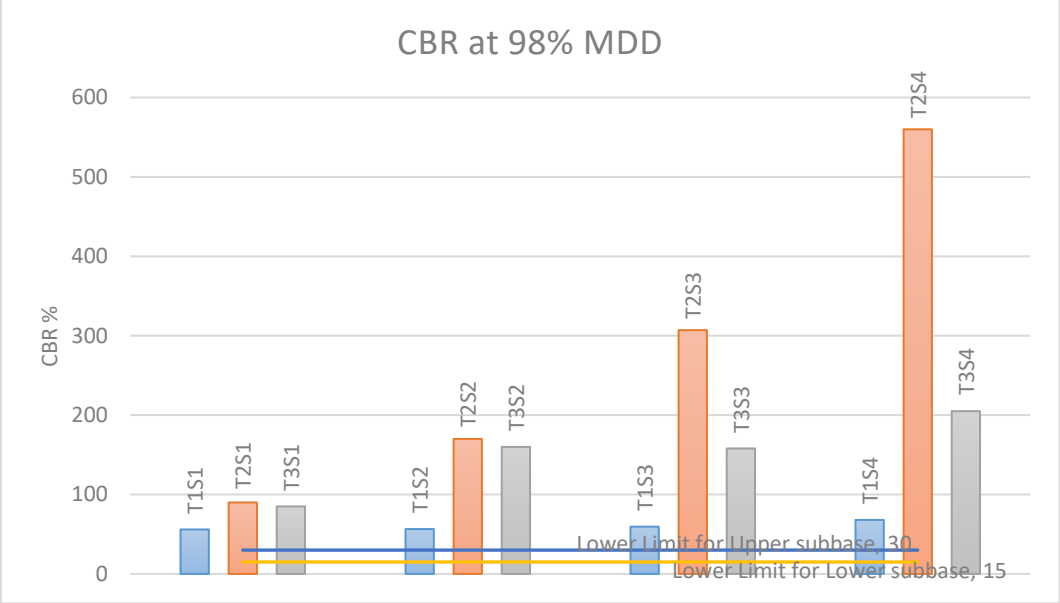

Figure 9: CBR at 98% MDD

4.5 Unconfined Compressive Strength test

To analyse the effect of cement on the strength of the C&D materials was analysed by conducting unconfined compressive strength test.

The unconfined compressive strength test results of gravel + RCA, gravel + CB and Gravel + RCA + CB blends with the addition of 5%, 6% and 7% cement are shown in Figures 10, 11 and 12 respectively.

The Gravel + RCA + CB exhibited the highest strength in all cases, with the same cement content and for the same curing duration, followed by RCA and CB.

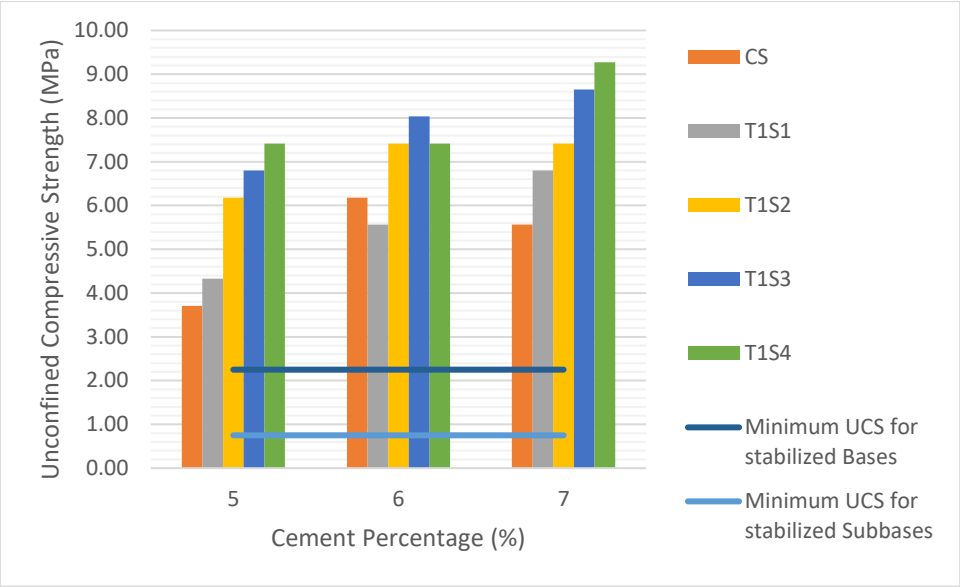

Figure 10: UCS results for Gravel + RCA series

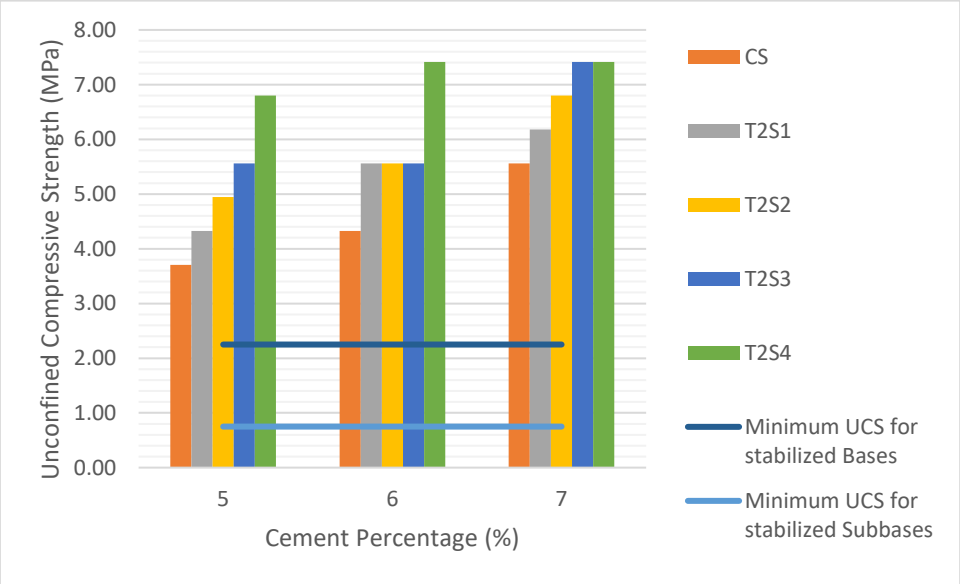

Figure 11: UCS results for Gravel + CB series

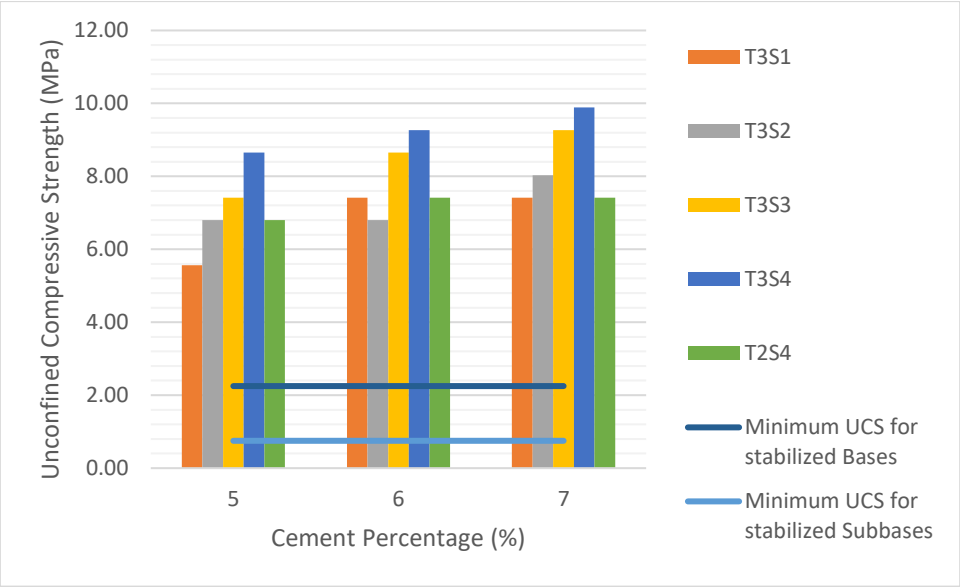

Figure 12: UCS results for Gravel + RCA + CB series

The UCS values as per the standards are 0.75-1.5 for stabilized road sub bases and 1.5-6.0 for stabilized road bases. Hence it can be seen from Figures 10, 11 and 12 that all the cement treated samples comply with standards for bases and sub bases.

5. CONCLUSION

Based on the outcomes of the study following conclusions can be drawn:

According to the results of particle size distribution, consistency tests, compaction test and CBR test, it can be concluded that with reference to the ICTAD specifications for road and bridge construction in Sri Lanka, proportions of C&D waste and gravel used were in conformity with the requirements to be used in embankments, lower sub base, upper sub base for flexible pavement construction as well as to be used as a road shoulder material. However, these samples did not meet with the consistency requirement of standard given for sub bases of Rigid Pavement since the observed values exceeded the maximum specified value of 25% Liquid Limit. Therefore the samples are considered not to be used in upper sub bases for rigid pavements.

In unconfined compressive strength test, it was observed a linear variation of the strength parameter with the increase of RCA and OPC percentage in the blend. The UCS values did satisfy with the local authority standards and hence cement treated construction and demolition waste is viable solution for road bases and sub bases of the country.

6. ACKNOWLEDGEMENT

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Design of an IoT System for Strain Measuring in Steel Structured Bridges

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Abstract – *In recent decades bridges have become an important economic icon in public and industrial transportation. As the condition of a bridge decides the safety of the users, continuous monitoring of structural condition is vital and it helps to establish an efficient transportation. Moreover, efficient measuring techniques help to make powerful decisions of its environment. Presently, the majority of railway bridges in Sri Lanka are steel structured bridges. Strain, vibration and movements measurements are the critical parameters used for measuring the condition of these steel structured bridges. Among them, strain monitoring plays a major role in determining the condition of bridges. Though strain gauge-based monitoring systems are being used for monitoring of strain, present monitoring practice in Sri Lanka is limited to analysis of pre-captured and stored strain data. This technique is obsolete and is associated with many drawbacks. Thus, we propose a continuous condition monitoring internet of things (IoT) system for steel railway bridges which directs to analysing the data online, hence having a better reliability. Through this design, a total integrated solution has been introduced to measure and analyse online strain data and to make fast decisions by the experts.*

Keywords*: Steel Structured Bridges, Strain Monitoring, Wireless Sensor Networks (WSNs), Internet of Things (IoT)*

1 INTRODUCTION

The transportation network of a country is one of the crucial factors, which has a direct impact to the country's economy. Services like transportation of raw material and then return with the products from the factories, passenger services etc. totally depend on the quality of the transportation network. Abreast of the time, due to the low maintenance of these infrastructure, they may be in a poor condition. Nevertheless, a simple accident caused due to poor conditioned transportation infrastructure may cause a serious loss even ranging to the loss of thousands of human lives. Therefore, conditions monitoring schemes of the transportation infrastructure are vital. On the other hand, the proper maintenance of the transportation infrastructure can be considered as a future investment to the country. When considering the efficiency of the transportation structures, bridges play an important role. Therefore, optimum conditions of the bridges are needed to be maintained for a safe and an efficient transportation. To maintain the optimum conditions throughout, bridges have to be designed together with good health monitoring mechanisms.

Presently, in Sri Lanka, the wired, manual monitoring systems are being used to monitor conditions of steel bridges [1]. Furthermore, the data is analysed offline. Here, a severe problem arises when the decisions with less accuracy are being obtained through

this system due to the obsolete and less accurate data, which has been obtained manually by the technical persons.
Therefore, considering the aforementioned circumstances, a wireless sensor network is introduced to overcome the above issues. Wireless sensor network is a dedicated sensor group which is networked with wireless communication infrastructure to gain some specific data without a human interference [2, 3]. The data is gathered, may be for monitoring purpose or may be to actuate some specific actuators.
In this paper, the strain is taken as the measured parameter of the system and the total sensor node consists of two major parts, namely the transducer and the communication unit.
The current manual strain monitoring system used in Sri Lanka is a wired monitoring system [1], which is consisting of a strain sensor element, data processing and acquisition unit (data logger) and the condition evaluation/decision making system. The sensor element is typically linked to copper cables. This traditional system is very expensive and when using for monitoring from a long-distance communication, most of the time it may be susceptible to disturbances. Another drawback of this system is that it doesn't possess any data encryption mechanism. As a solution, we present the design of a wireless sensor network (WSN) based internet of things (IoT) system for measuring strain in steel railway bridges.
The rest of the paper is organized as follows. Section 2 further discusses the currently available WSN based monitoring systems around the world. In Section 3, we present the proposed IoT strain measuring system. In Section 4, we further elaborate on the functionality of the proposed strain measuring system followed by the prototype implementation details and the testing results. Finally, Section 5 concludes the paper highlighting several future research avenues.

2 WSN-BASED MONITORING SYSTEMS

There are a number of bridge strain monitoring systems employed around the world. In the system of [4], an accelerometer is deployed for strain monitoring and the accelerometer reading is taken over the three directions XYZ. Here the accelerometer reading is further enhanced by a signal conditioner. Eventually it is transmitted via a wireless module. Through this method, acceleration parameter values can be directly obtained and those parameters can also be directly used to calculate the strain. It also helps to monitor the movements of the bridges. Nevertheless, its durability is comparatively low compared to strain gauges. Moreover, it uses complex mathematical calculations when determining strain values from the data collected at a particular node [4].

To overcome the above mentioned drawbacks, a wireless strain monitoring system for raw strain measurement is proposed in [5]. This system consists of a micro-electromechanical system, which has an inbuilt data processing unit within the same sensor elements. This consumes a very low power and provides high efficiency as the on-board microprocessor of the wireless sensor can facilitate an efficient distributed data processing in real time. Furthermore, its durability is high as it uses fiber optic strain measuring sensors.
In general, even the bridge monitoring systems as in [5] have to overcome many challenges as listed below.
As the fact that strains of a bridge is generally small, sensor output signal would also be in a very small scale of Volts. Therefore, small fluctuations of the signal is needed be detected by the signal processing unit. Meantime, high resolution of the sensor plays a vital role. On the other hand, when a locomotive is moving on a bridge, it causes to vary the strain continuously. In order to capture the corresponding sensor output voltage variation, high

frequency processing system is needed. Generally the railway bridges are in high noisy environments, hence the signal to noise ratio at the sensor output needs to be improved. Another crucial requirement of the sensor node is the high speed data processing and transmission. Processed data should be transmitted with a high speed. Due to the low power availability at the sensor, it cannot maintain a large buffer or memory to store processed data. At the same time, nowadays, low-power radio transceivers provide a limited bandwidth (a maximum of 250 kbps in the case of IEEE 802.15.4 networks). Time synchronization is also a crucial factor in WSNs. Since each sensor node has its own time clock, sensor and relay nodes must be synchronized with each other, in order to eliminate phase delays.
To overcome all the aforementioned challenges and to cater for all these requirements, in this research we propose a reliable and efficient WSN based strain monitoring IoT system for steel structured railway bridges, which is much superior over the systems in [4, 5] .

3 DESIGN METHODOLOGY

The proposed WSN based IoT steel bridge monitoring system consists of several important sub units. Amongs them the most vital is the wireless sensor network and its nodes which captures the distributed strain measurements. The structural construction of a sensor node is depicted in Figure 1.

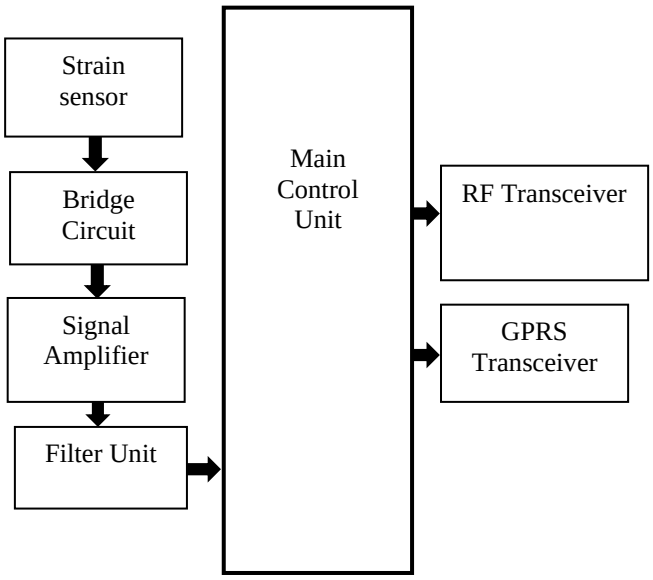

Figure 1: System block diagram of a sensor node

As mentioned earlier, in this design strain is the measured parameter, strain gauge is used as the sensor element which is effectively a variable resistor whose resistance varies with the strain applied. Sensor node is consisted of several sub units including sensor element, filter unit, signal conditioning unit, ADC and processing unit. When a strain is applied on the strain gauge, it changes its resistance which in-turn creates a voltage difference at the bridge output. This output voltage difference is directly proportional to the change of the variable resistance in the strain gauge, hence to the strain applied.

3.1 Sensor Element and Wheatstone Bridge Circuit

A strain gauge of 10mm was selected as the sensor element due to its considerable resistance change possible, the temperature factors and also its availability. Here, the sensor element shown as R5 is converted in to a transducer by plugging in to a one branch of the Wheatstone bridge [6]. In order to reduce the current flow through the branches of the bridge, an additional resistor R1 was attached as shown in figure 2.

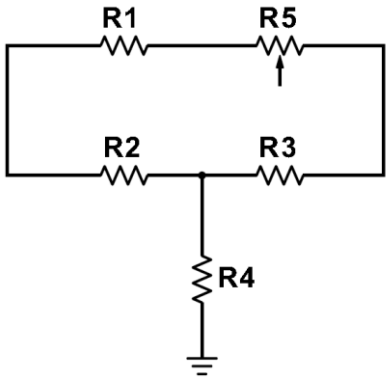

Figure 2: Basic sensor element placement in a Wheatstone bridge

The calibration and the scaling were carried out using (1) [6].

$$\frac{V_o}{V_{ex}} = \frac{GF.\varepsilon}{4}\left\{\frac{1}{1+GF.\varepsilon/2}\right\} \qquad \text{--------(1)}$$

Where,

$\boldsymbol{V_o}$- Sensor output signal.

$\boldsymbol{V_{ex}}$- Input voltage of the bridge.

$\boldsymbol{GF}$- Gauge factor of strain gauge.

$\boldsymbol{\varepsilon}$- Strain.

The gauge factor can be calculated as (2).

$$GF = \frac{\Delta R/R}{\Delta L/L} = \frac{\Delta R/R}{\varepsilon} \qquad \text{--------(2)}$$

It is very important to note that the calculated parameter values and the measured values of GF may slightly change due to the temperature coefficient of the strain gauge and due to the physical condition of the resistors. In this work, we obtain the GF value through calculations.

3.2 Amplifier Unit

As stated earlier, the voltage difference at the Wheatstone bridge output is directly fed to the amplifier unit. INA 114 instrumentation amplifier [7] was selected as the amplifier unit as it consumes very low current and also due to its law noise amplification capability. Moreover, it supports an input signal with high impedance and produces an output with low impedance.

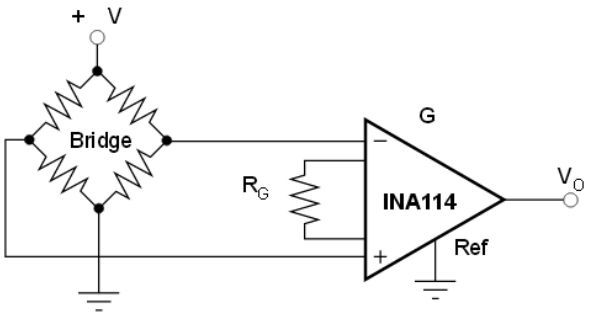

Figure 3: INA 114 low noise amplifier connection to bridge output

3.3 Filter Unit

It is important to note that the sensor node has to work in a very noisy outdoor environment. On top of that, the output of the transducer is usually a few millivolts. Therefore, noise can be easily added to the signal and distort the output. Therefore the transducer output is first amplified. In order to filter out the noise in the captured signal, an active low pass filter was used after the amplifier output.
Another important aspect is the stability of the filter unit, which totally depends on the quality of the passive elements. In this work we design the unity gain filter using MCP601 low noise operational amplifier [8]. Cut-off frequency can be adjusted by varying the resistor and capacitor values in the circuit of Figure 4. Note that one of the demarcating features of this filter unit is, it is an anti- alliancing filter, which directly helps to filter out the noise.

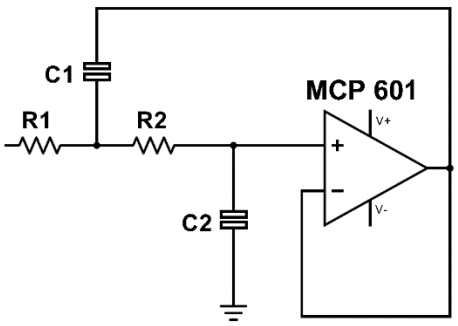

Figure 4: Active low pass filter topology

3.4 Analog to Digital Convertor (ADC) and Processing Unit

ARDUINO UNO microcontroller board was employed as the main signal processing unit. This controller consists of a ten bit in-built ADC. Therefore, a $\frac{1}{1024}$ digital resolution is provided by the ADC.

3.5 Basic Architecture of the Wireless Sensor Network

The basic system architecture of the network model consists of two major sub systems namely, sensor network and the server network. In the sensor network, the sensor nodes

capture the strain values at each point and relay these data to a central node which we name as master node. The sever network consists of the master node and the linkage to the external remote networks in point-to-point mode. We use radio frequency (RF) communication for the sensor network while using general packet radio service (GPRS) based communication in the server network. The operating algorithm of Sensor nodes is depicted in figures 5 and 6. Also, figure 7 presents the communication algorithm for the master network.

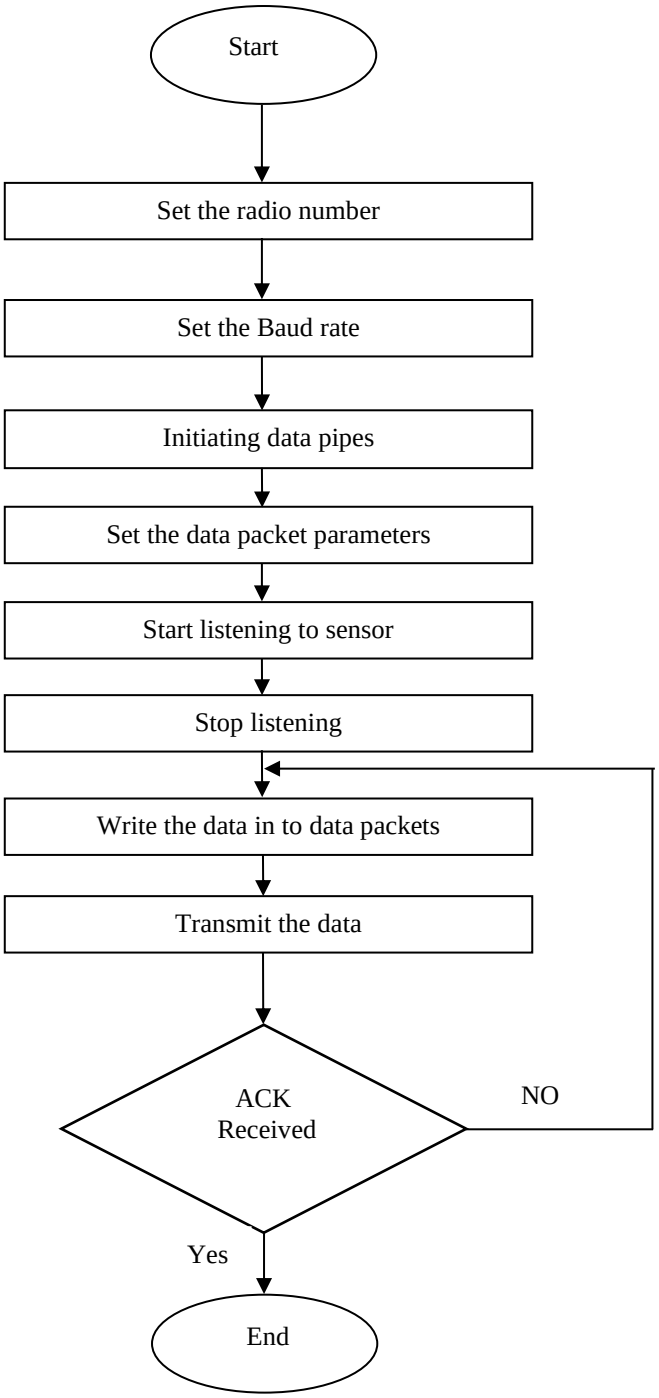

Figure 5: RF communication algorithm (Transmit)

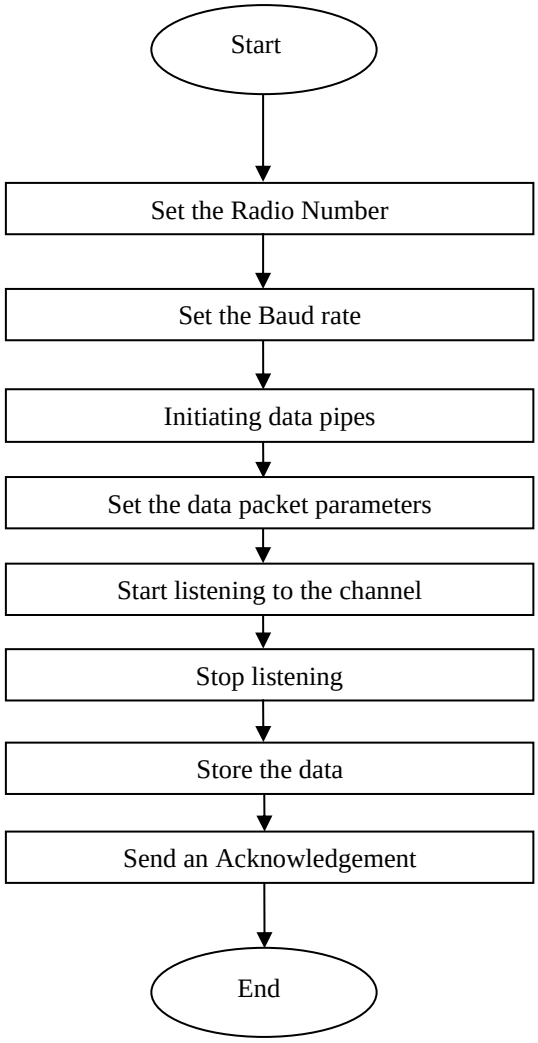

Figure 6: RF communication algorithm (Receive)

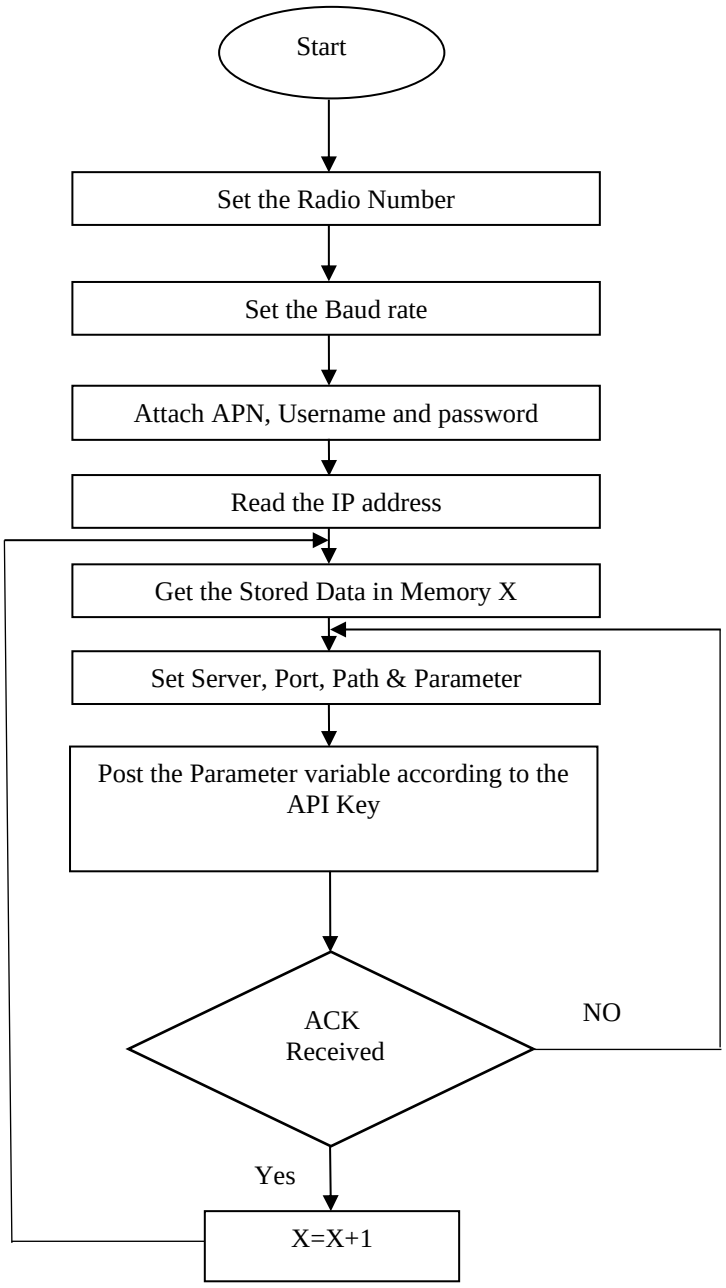

Figure 7: Master network communication algorithm

Recall that, master node to server communication is established through GPRS communication. One of the major advantages of using GPRS is the ability to achieve non-line of sight, long distance communications. The real time data which is obtained by the sensor network is ultimately transferred to an IoT server. Here the THINGSPEAK IoT server is used for that particular test implementation process. Figure 8 depicts the data reception and storage at the master node.

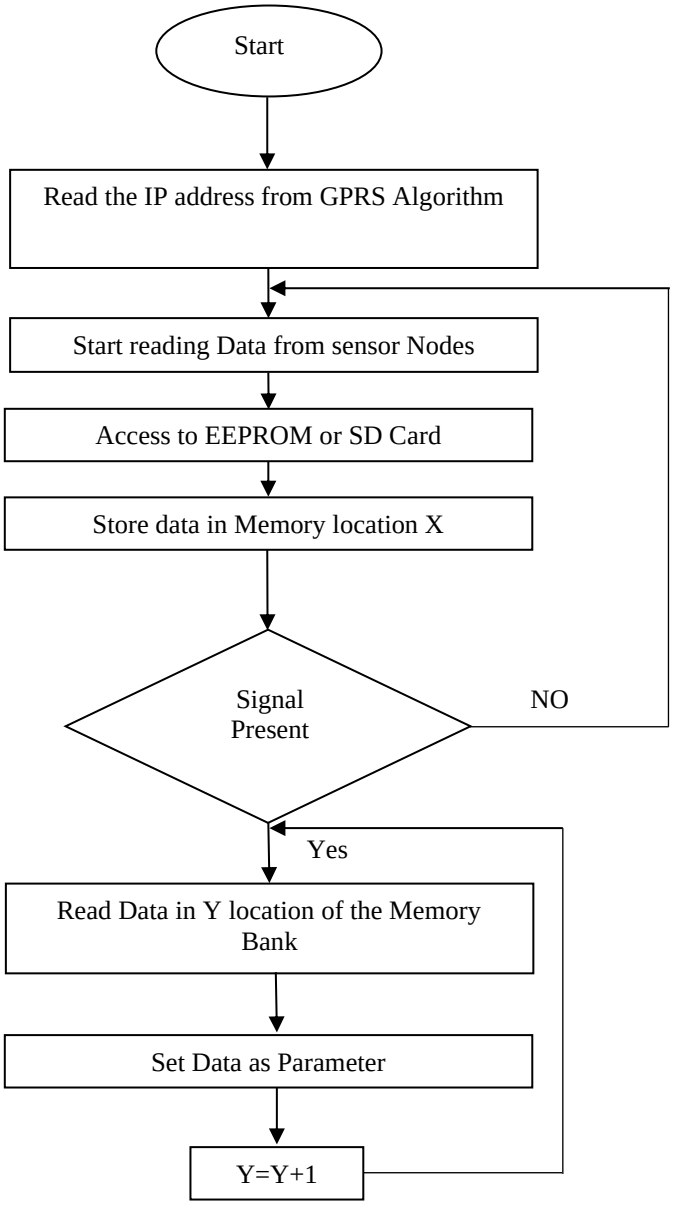

Figure 8: Data storage algorithm

5 OPERATION, PROTOTYPE IMPLEMENTATION, TESTING AND RESULTS

The overall system architecture is illustrated in figure 9. As mentioned earlier, THINGSPEAK IoT server is used to facilitate a graphical user interface (GUI) at the remote server. Trend of the strain pattern can be easily visualized and strain data can be downloaded in excel format for further analysis using the data available at the IoT server.

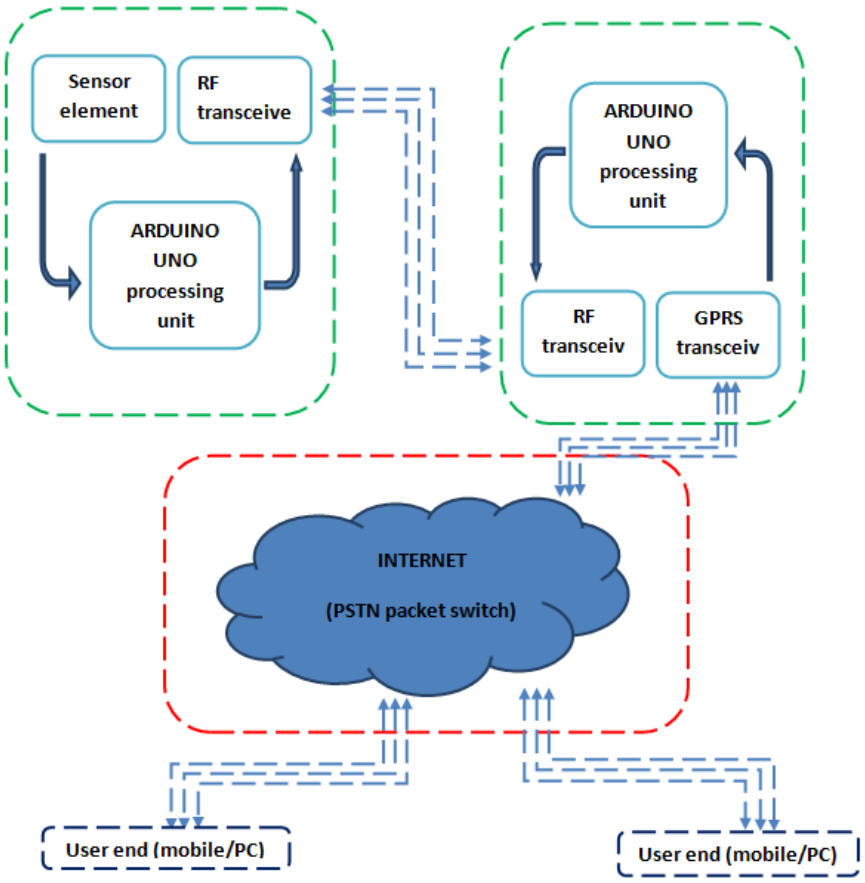

Figure 9: Overall System Architecture

A prototype system was implemented and initially the response of the sensor element was obtained by varying the load, in order to check its linearity. Table 1 shows the output voltage value of the sensor versus the applied load to cause strain. Graphical representation in Figure 10 clearly depicts a linear behavior.

Table 1: Sensor Element's Linearity Test

Load (kg)	V_{out} (mV)
0	0.912
20	0.990
30	1.032
40	1.074
50	1.112
75	1.210
100	1.311
125	1.414
150	1.513

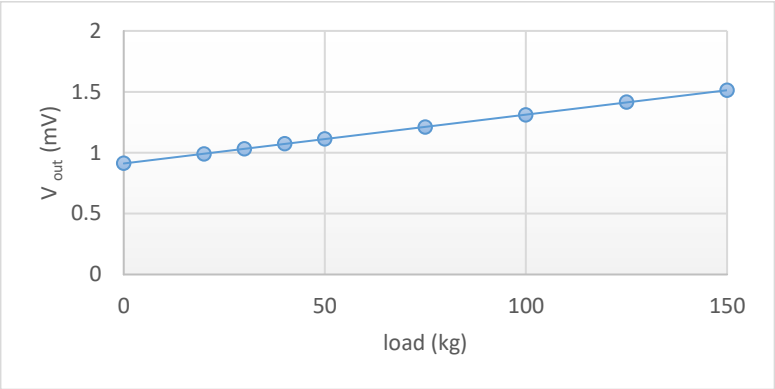

Figure 10: Linearity of the Sensor

Another test was performed to determine the effect of interference signals at a sensor node, especially present when a metal train moves over the railway track. The interference test was done under the effect of minimum magnetic field. The tabulated data for the interference test is shown in the figure 11. Number of data received was taken through the mean value of three different attempts. An optimum condition for maximum data receiving was obtained at 9600 baud rate with 100ms delay between two data sets. The correctly received number of data packets is a clear indication of the effect of external interference. Thus, we can conclude that even under interference, data packets can be effectively transmitted at very high data rates maintaining more than 50% success rate. Moreover, transmitting at lower rates and larger accepted delay times can yield higher success rates exceeding 75%. This is very much adequate for our IoT system application.

Table 2: Interference Test data

No. of data in data set	Delay time (ms)	Baud rate	No of data received
100	1000	115200	51
100	100	115200	55
100	10	115200	50
100	1000	9600	81
100	100	9600	96
100	10	9600	77
100	1000	4800	67
100	100	4800	76
100	10	4800	69

It is also worth to investigate the maximum separation between the nodes in the WSN to maintain an effective relaying of data packets generated at each sensor node, amidst all interference. Table 3 tabulates the RF communication data in the presence of a S12 locomotive engine's interference. Here, the number of data received was obtained

through the mean value of three different attempts. It can be observed that the optimum distance in-between the two sensor nodes to have a sufficient success rate, is around 2.5m.

Table 3: RF Data near the S12 locomotive engine

No. of data in the test set	Distance between two sensor nodes(m)	No. of data received
100	3	58
100	2.75	64
100	2.5	78
100	2	79
100	1.75	80
100	1.5	84
100	1.25	87
100	1	86

Moreover, another test was carried out to determine the success of the server network communication under the created electromagnetic interference, due to the moving locomotive engine. The test results are tabulated in Table 4, under different baud rates. It is clear that the communication achieves a success rate exceeding 95%.

Table 4: GPRS Test Data

No. of data	No. of connection failures	ACK failures	Baud rate	No. of data received to server
50	2	3	115200	48
50	3	1	115200	49
50	1	1	115200	49
50	6	2	115200	44
50	1	2	9600	48
50	1	2	9600	49
50	2	0	9600	48
50	1	1	9600	49
50	2	0	4800	48

Furthermore, figures 11 and 12 show the graphical display of data in the web interface of the THINGSPEAK IoT server.

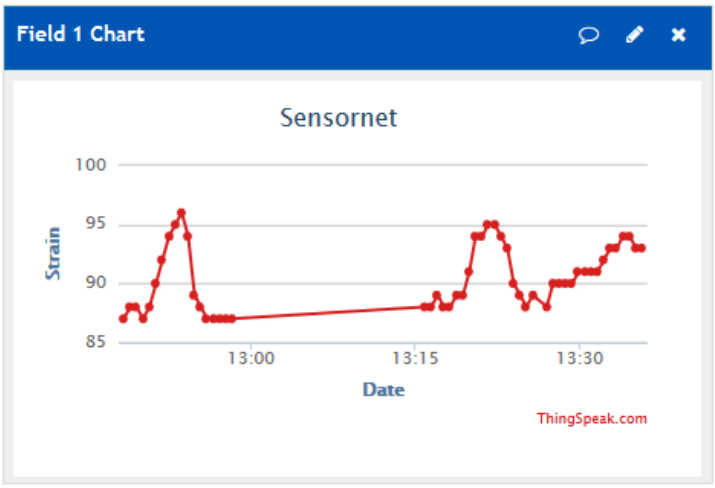

Figure 11: The graphical representation of the Strain

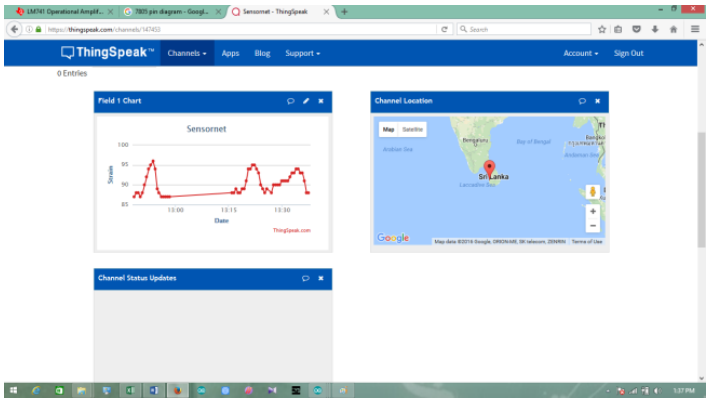

Figure 12: Graphical User Interface

6 CONCLUSION AND FUTURE WORK

Nowadays, IoT has become a vital platform, which provides an easier approach to bring loads of information to one's fingertips. If a technical personnel can access the strain data of steel structured bridges, measured via the proposed IoT system from anywhere in the world, that would be a huge advantage for minimizing the bridge infrastructure failures. Therefore, it directly helps to increase the total productivity and also to enhance the safe transportation conditions.

This research basically focused on enhancing the condition of steel structured bridges in Sri Lanka. Furthermore, it is introduced as an IoT product of low cost, long life and with minimum maintenance.

Design and implementation of hardware to withstand extreme environmental conditions was beyond the scope of this work, hence we categorize the same under future work.

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Morphological interrelationship of selected economically important Capsicum spp in Sri Lanka

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Abstract – *The genus Capsicum belongs to the family Solanaceae has five domesticated species, C. annuum, C. baccatum, C. chinense, C. frutescens, and C. pubescens, and more than thirty wild species. Knowledge of the local Chilli (Capsicum spp) varieties and minor scale growing Capsicum species; specially the 'Kochchi' group as well as wild species, are not properly described, evaluated and documented. This constitutes has created a major research gap by not having a proper understanding specially genetic constituents of the minor scale growing species as well as wild species. As a basic solution to this problem, the present study was conducted to study the interspecies relationships using morphological characterization. A total of twenty-seven (27) accessions from the Plant Genetic Resources Center, Gannoruwa, and Peradeniya were morphologically characterized in the present investigation. They corresponded to eighteen (18) Capsicum annum; 'Miris' accessions, eleven (11) Capsicum frutescens; 'Kochchi'. All five plants grown from each accession were subjected to morphological characterization using the Manual for Capsicum morphological characterization developed by the Plant Genetic Resource Center in 1999. Morphological data were recorded for 38 characters corresponding to 27 qualitative and 11 quantitative traits. Morphological data were computed to calculate the means for each of the accessions. A phenogram was generated using the Numerical Taxonomic System, Mesquite software. The phonogram generated displayed morphological diversity among Capsicum accessions by separating the entire phylogeny into four (04) major clades, whereas with two individual clades, 1 and 2; C. annuum 557 and C. annuum 476. The third clade indicates interrelationships of nine Capsicum frutescens accessions namely 391, 762, 11641, 1070, 9089, 1083, 11642, and 11644. Capsicum annuum; 12829, 255, and 388 accessions nested in the second clade by closely associated with the Capsicum frutescens accessions indicating Capsicum annuum accession umber 12829 as the common ancestor. However, this relationship should be further studied through molecular phylogenetical analysis. Clade number four (04) is further divided into two clades separating one cluster only for Capsicum annuum accessions; 180, 5152, 1223, 819, 1782, 1781, 1780, and 1778. The present study does not reveal the morphological phylogeny entirely of the studied Capsicum accessions. Although morphological characterization is recognized as the starting point in the assessment of genetic diversity, morphological markers should be complemented with molecular markers for more advanced genetic diversity study in Capsicum.*

***Keywords**: Morphological characters, Capsicum annuum, Capsicum frutescens, phonogram*

1.0 INTRODUCTION

The genus *Capsicum* consists of all the 'chili', 'pepper' plants, and the confusing terminology 'chilli' is often used frequently and interchangeably with other names globally including 'chile', 'aji', and 'paprika' to refer to multiple species (Basu and De, 2003). The genus Capsicum has been cultivated since at least 6000 B.C in America. The genus has five domesticated species, *C. annuum, C. baccatum, C. chinense, C. frutescens*, and *C. pubescens*, and more than thirty wild species (Carrizo Garcia et al, 2013; Heiser and Pickersgill, 1969). The most commonly cultivated Capsicum species is *C. annuum*, which is domesticated in northern Latin America (Pickersgill, 1971; Kraft et al, 2014). *C. chinense* and *C. frutescens* are domesticated in tropical northern Amazonia, while *C. baccatum* and *C. pubescens* are more prevalent in Latin America and mid-elevation southern Andes, respectively (Pickersgill, 1971) The domesticated species can be classified by morphological traits: seed color, corolla yellow spot, number of flowers per axil, calyx annular constriction, and flower position (IBPGR, 1983).
In Sri Lanka we refer to all the Capsicum as '*Miris*' and differences identify with either the shape or the color of the pods. We do not have specific names for hundreds of species, cultivars or varieties we cultivate or see in our surroundings. The number of global species within the *Capsicum* genus has long been subject to debate, with various authors ascribing 25 species to the genus, 33 by Morrison in 1680, 27 by Tournefort in 1700, two by Linnaeus in 1753, and five by Smith and Heiser, 1951 (Basu and De, 2003). There are presently considered to be five domesticated species of *Capsicum* from approximately 25 recognized species in the genus, the primary distinguishing characteristics being flower and seed colour, the shape of the calyx, number of flowers per node, and their orientation; these five species are *C. annuum, C. frutescens, C. chinense, C. baccatum* and *C. pubescens* (Hawkes et al., 1979; Basu and De, 2003; Aguilar-Melendez et al., 2009). Cultivated *C. annuum* is thought to have been domesticated from wild populations of *C. annuum* var. *glabriusculum* in Mexico, possibly multiple times from geographically separate wild populations (Aguilar-Melendez et al., 2009).

Sri Lanka has a number of Capsicum species that are popular among consumers as well as unpopular species grown mainly at the village level. Most of these species are underutilized having their own aroma, pungency, taste, and flavor. Further, some of these varieties show resistance to the pests and diseases and are specially resistant to the current scenario of environmental and climatic changes. Among commonly grown species five domesticated species are *Capsicum annum* L, *C. frutescens* L, *C. chinense* Jacq., *C. baccatum* L., *C. pubescens* Ruiz and Pavon. *C. annuum* L. is the most commonly cultivated either for pungent fruited genotypes called Chilli or '*Miris*' or non-pungent fruited genotypes called sweet pepper (syn. capsicum, paprika, bell pepper etc.) (Sanjay et al., 2009). Some *Capsicum* spp (syn. Kochchi, Nai Miris, Hini Miris etc.) is having high pungency and different aroma as well as taste and flavour, preferred by most the Sri Lankans. However, minor scale growing these *Capsicum* spps. known as '*Kochchi*' is underutilized in Sri Lanka and most popular as the home garden crop.

1.1 Research rationale

The ability to characterize morphological diversity is indispensable for effective management and sustainable use of Capsicum genetic resources in breeding programmes. Primary characterization involves measuring simple plant characters that can be easily recorded through visual observations at different plant growth stages (leaf area, fruit shape, size and colour, plant prickliness, and hairiness). Secondary characterization deals with more complicated morphological traits of agronomic importance such as pest and disease resistance, fruit set, yield potential, and biochemical (glycoalkaloid or antioxidant) properties (Ayad, 1995). Therefore, morphological crop descriptors allow quick and easy discrimination between phenotypes. They are generally highly heritable traits that can be easily recorded through visual observations and that are equally expressed in all environments.

Knowledge of the local Chilli (*Capsicum* spp) varieties and minor scale growing Capsicum species; specially the '*Kochchi*' group as well as wild species, are not properly described, evaluated and

documented. This constitutes a major research gap since minor scale growing Capsicum species; such as '*Kochchi*' group is currently underutilized. Further, there is no proper understanding of the wild species of Capsicum. Enhancement and protection of the local species or varieties on the other hand require analysis of their characteristics, diversity and relationship with similar accessions. There is an urgent need for the active reintroduction of Capsicum genetic diversity resources into the current production system due to various reasons. First, the use of *Capsicum* spp effectively with improved varietal compositions among Sri Lankans is required to optimize their use and also protect the existing local species and cultivars from extinction. Secondarily, overall research must be diverted to raise improved varieties with the economical values specially genetically divert or adjusted improved varieties such as, to resist pests and diseases, changing climate, to acquire high yield, to develop new varieties with consumer referable pungency, taste, and aroma.

Understanding genetic relationships are possible using morphological characterization as well as molecular characterization of Capsicum. Further, understanding the genetic resources will also enable researchers to identify accessions with desirable traits, monitor their genetic stability and integrity and screen for duplicate accessions to minimize waste of resources and lower management costs. Morphological descriptors for *Capsicum annuum* L, *C. frutescens* L, *C. chinense* Jacq., *C. baccatum* L., *C. pubescens* Ruiz and Pavon. *C. annuum* L. has been developed by Plant Genetic Resource Center (PGRC), Gannoruwa which provides internationally accepted definitions for these descriptors and includes complete information on important quantitative and qualitative traits illustrated by figures and measured either in metric or arbitrary scale. To this end, the present research study was conducted to access the morphological diversity of Capsicum accessions under *in-situ* conservation at PGRC, Gannoruwa, Sri Lanka. Therefore, the objective of the research is to initiate morphological characterization of Capsicum genetic resources for a basic understanding of the genetical interrelationship among species and varieties which will be helpful for future evaluation, monitoring, and documentation.

2.0 MATERIALS AND METHODOLOGY

2.1 Planting Material

A total of twenty-seven (27) accessions from the Plant Genetic Resources Center, Gannoruwa, and Peradeniya were morphologically characterized in the present investigation. They corresponded to eighteen (18) *Capsicum annum; 'Miris'* accessions, eleven (11) *Capsicum frutescens*; '*Kochchi*'. These accessions are maintained as *in-situ* collections at the Plant Genetic Resource Center. Among the 27 Capsicum accessions, nine accessions were collected from the Intermediate Zone of Sri Lanka while the remaining accessions were collected from different agro-ecological regions in Sri Lanka as listed in Table 2.

2.2 Experimental site

The experiment was conducted at the Open University of Sri Lanka, Nawala, Nugegoda to ensure that all Capsicum accessions were at the same stage of growth for morphological characterization. The experiment site received a mean annual rainfall of <800 mm and had suitable agro-climatic conditions favorable for the growth and development of eggplant crops with successful expressions of all traits.

2.3 Establishment and maintaining pot plants

Nurseries were prepared inside the insect-proof plant houses. Seeds were sown on separate plastic trays 30 cm in length and 15 cm in width and 10 cm in depth separately. Before seed sowing, hot water treatment was performed by soaking seeds in a hot water bath at 50°C for 25 minutes followed by fungicide dressing with Captan to prevent any incidence of seed and soil borne diseases. Three weeks after seed sowing, the plants were transferred to the 1 feet diameter pots

filled with 1:1:1:1 – Top soil : sand : cow dung : coir dust potting mixture. One plant per accession with 5 replicates was grown such that 5 plants per each accession with a total number of 135 plants from all the 27 accessions were studied. Transplants, management, and cultural operations were carried out as per the recommendations of the Department of Agriculture. All cultural practices in terms of pest and disease control, applying fertilizer, and watering were followed according to the recommendations of the Department of Agriculture. One month after transplanting, the plants were covered with insect-proof net bags, 90 cm in width and 120 cm in height to avoid cross-pollination. Self-pollination was carried out to produce seeds for future research.

Table 01: Accessions received from Plant Genetic Resource Center

Accession Number	Common/Accession name	Scientific name	Collected location
000762	Sudukochchi	*Capsicum frutescens*	Mathale
01783	Malumiris	*Capsicum annum*	Kurunegala
01782	Malumiris	*Capsicum annum*	Kurunegala
01781	Malumiris	*Capsicum annum*	Kurunegala
01780	Malumiris	*Capsicum annum*	Kurunegala
01778	Malumiris	*Capsicum annum*	Kurunegala
09089	Kachchi	*Capsicum frutescens*	Rathnapura
10173	Miris	*Capsicum frutescens*	Sri Lanka
10170	Miris	*Capsicum frutescens*	Sri Lanka
11644	Kola kochchi	*Capsicum frutescens*	Sri Lanka
11642	Dam kochchi	*Capsicum frutescens*	Sri Lanka
11641	Kochchi	*Capsicum frutescens*	Sri Lanka
00255	Miris	*Capsicum annum*	Hambanthota
00476	Miris	*Capsicum annum*	Nuwara Eliya
12830	Malumiris	*Capsicum annum*	MI (FCRDI)
12829	Wannimiris	*Capsicum annum*	MI (FCRDI)
12935	Malumiris	*Capsicum annum*	Trincomalee
00388	Hen miris	*Capsicum annum*	Kurunegala
1223	Malumiris	*Capsicum annum*	Anuradhapura
1385	Malumiris	*Capsicum annum*	Badulla
5152	Malumiris	*Capsicum annum*	Gampola
557	Miris	*Capsicum annum*	Nuwara Eliya
00819	Miris	*Capsicum annum*	Galle
00180	Miris	*Capsicum annum*	Hambanthota
00193	Miris	*Capsicum annum*	Hambanthota
00391	Sudukochchi	*Capsicum frutescens*	Kurunegala
00840	Kochchi	*Capsicum frutescens*	Galle
1070	Kochchi	*Capsicum frutescens*	Kegalle
1083	Heenkochchi	*Capsicum frutescens*	Kegalle

2.4 Morphological Characterization

All five plants grown from each accession were subjected to morphological characterization using the Manual for Capsicum morphological characterization developed by the Plant Genetic Resource Center in 1999. Morphological data were recorded for 38 characters corresponding to 27 qualitative and 11 quantitative traits (Tables 2 and 3.). Each of the accession for these characteristics was determined for all 38 characters for all thirty-eight descriptor states were recorded.

The morphological traits recorded for the different Capsicum accessions were entered into an Excel datasheet and were prepared to document the above-mentioned groups of plant characters. Morphological data is computed to calculate the means for each of the accessions. A phenogram was generated using the Numerical Taxonomic System, Mesquite software (Za, 2014). Thirty-eight standardized qualitative and quantitative traits were subjected to generate the phenogram.

3.0 RESULTS AND DISCUSSION

The morphological differences between large-scale cultivated Capsicum (Chilli) are easily discerned. All underutilized forms of chilies have small, red, berry-like fruits with colors and sizes attractive to birds. Further, they had various fruit shapes. Some domesticated species have deciduous fruits, which, if not eaten by birds, fall to the ground while the seeds are still at peak viability, while some exhibit variable fruit and flower coloration (designed to appeal to the human

Table 2: Quantitative morphological descriptor states measured in an arbitrary scale; codes for traits and description of the scale used in morphological diversity study of Capsicum collections listed

Quantitative traits	Range (scale)
Plant height	1 - 5 (1 = short~ < 25, 2 = 25 - 45, 3 = 46 - 65, 4 = 66 - 85, 5 = very large ~ > 85)
Mature Leaf length	Not coded (Same stage as in 10 cm - Average of 10 leaves)
Mature Leaf Width	Not coded (Same stage as in 10 cm -Measured on the widest part of the leaf use same leaves as in 13)
Days to flowering	Not coded (50% of plants have at least one open flower)
Number of flowers per axil	1 = 1, 2 = 2, 3 = 3 / more, 4 = many flowers in bunches but each in individual axil, 5 = other (cultivars with two flowers in first axil and with one only in the other)
Fruit length	Not coded (Average fruit length of 10 ripe fruits of the second harvest)
Fruit width	Not coded (Average fruit width of 10 ripe fruits of the second harvest)
Fruit Weight	Not coded (Average fruit width of 10 ripe fruits of the second harvest)
Fruit wall thickness	Not coded (Average fruit width of 10 ripe fruits of the second harvest, measured at the point of maximum width to one decimal point)
1000 Seed Weight	Not coded (100 x 10)
Number of Seeds per fruit	1 - 3(1 = < 20, 2 = 20 – 50, 3 = > 50)

Table 3. Qualitative morphological descriptor states measured in an arbitrary scale; codes or traits and description of the scale used in morphological diversity study of Capsicum collections listed

Qualitative traits	Range (scale)
Hypocotyls Colour (recorded when terminal bud is 1 – 2 mm in size)	1 - 3 (1 = white, 2 = green, 3 = Purple)
Cotyledons leaf colour	1 - 9 (1 = light green, 2 = green, 3 = dark green, 4 = light purple, 5 = purple, 6 = dark purple, 7 = variegated, 8 = yellow, 9 = other)
Life Cycle	1 - 3 (1 = annual, 2 = biennial, 3 = perennial)

Stem Colour	1 - 4 (1 = green, 2 = green with purple stripers, 3 = purple, 4 = other)
Nodal Anthocyanin (Recorded at plant maturity in whole plant)	1 - 7 (1 = green, 3 = light purple, 5 = purple, 7 = dark purple)
Stem Pubescence (Recorded on mature plants, excluding the first two nodes below the shoot)	3 - 7 (3 = sparse, 5 = intermediate, 7 = dense)
Plant growth habit (Observed when 50% of the plants bear ripe fruits)	3 - 9 (3 = prostrate, 5 = intermediate, 7 = erect, 9 = other)
Branching habit	3 - 7 (3 = sparse, 5 = intermediate, 7 = dense)
Leaf colour (Recorded when in 50% of plants the first fruit has begun to ripen - 10 leaves on the main branches)	1 - 8 (1 = yellow, 2 = light green, 3 = green, 4 = dark green, 5 = light purple, 6 = purple,7 = variegated, 8 = other)
Leaf shape	1 - 3 (1 = deltoid, 2 = Ovate, 3 = lanceolate)
Leaf pubescence	3 - 7 (3 = sparse, 5 = intermediate,7 = dense)
Calyx margin	1 - 4 (1 = entire, 2 = intermediate, 3 = dentate, 4 = other)
Calyx annular constriction	0 - 1 (0 = absent, 1 = Present)
Anthocyanin spots or stripes on the fruit	0 - 1 (0 = absent, 1 = Present)
Fruit color at intermediate stage	1 - 8 (1 = white 8 = other)
Fruit Set	3 - 7 (3 = low, 5 = intermediate, 7 = high)
Fruit colour at mature stage	1 - 13 (1 = white, 6 = orange, 8 = red, 10 = purple, 12 = black, 13 = other)
Fruit shape	1 - 6 (1 = elongate, 2 = round,3 = triangular, 4 = campanulate, 5 = blocky, 6 = other)
Fruit shape at pedicel attachment	1 - 5 (1 = acute, 2 = obtuse, 3 = truncate, 4 = cordate, 5 = lobate)
Neck at base of fruit	0 - 1 (0 = absent, 1 = present)
Fruit shape at the blossom end	1 - 5 (1 = pointed, 2 = blunt, 3 = sunken, 4 = sunken and pointed, 5 = other
Fruit blossom end appendage	0 - 1 (0 = absent, 1 = Present)
Fruit cross-sectional corrugation (1/3 from pedicel end)	3 - 7 (3 = slightly corrugated, 5 = intermediate, 7 = corrugated)
Fruit surface	1 - 3 (1 = smooth, 2 = semi wrinkled, 3 = wrinkled)
Fruit pungency at maturity	0 - 6 (0 = not, 3 = low, 5 = intermediate, 6 = high)
Seed colour	1- 4 (1 = straw, 2 = brown,3 = black,4 = other
General uniformity of the accession	1 - 3 (1 = uniform, 2 = variable, 3 = highly variable)

eye); gigantism of the fruits, seeds, flowers, and leaves (Cochran, 1940; Eshbaugh, 1976); and retention of the fruit on the peduncle at maturity (Pickersgill, 1969; Eshbaugh, 1976).

When early taxonomists compared various Capsicum taxa, they noted that chilies were sorted into two distinct groups: one typified by small, red fruits and the other by large fruits. This classification effectively separated the wild and domesticated forms of Capsicum but bore no relevance to evolutionary relationships.

The thirty-eight quantitative and qualitative descriptor states characterized displayed a high level of morphological diversity among Capsicum accessions (Figure 01). The range of variation for the different quantitative descriptors revealed wide variability in all the quantitative descriptors studied. Numerical comparisons of morphological traits (Cochran 1940; Eshbaugh 1970; Jensen et al. 1979; Pickersgill et al. 1979) and cytogenetic analyses (Shopova 1966; Ballard et al. 1970; McLeod et al. 1979a, 1979b, 1982; Moscone et al. 1993) have been used to resolve relationships from the past. The numerical analyses typically included a limited number of species and focused primarily on the relationships of cultivated varieties to their wild progenitors. The numerical analyses of thirty-eight qualitative and quantitative morphological characters of the twenty-seven accessions resulted in the phonogram in Figure 01. The phonogram indicated four prominent clades with two individual clades, 1 and 2; *C. annuum* 557 and *C. annuum* 476. The third clade indicates interrelationships of nine *Capsicum frutescens* accessions namely 391, 762, 11641, 1070, 9089, 1083, 11642, and 11644. *Capsicum annuum;* 12829, 255, and 388 accessions nested in the second clade by closely associated with the *Capsicum frutescens* accessions indicating *Capsicum annuum* accession umber 12829 as the common ancestor. However, this relationship should be further studied through molecular phylogenetical analysis.

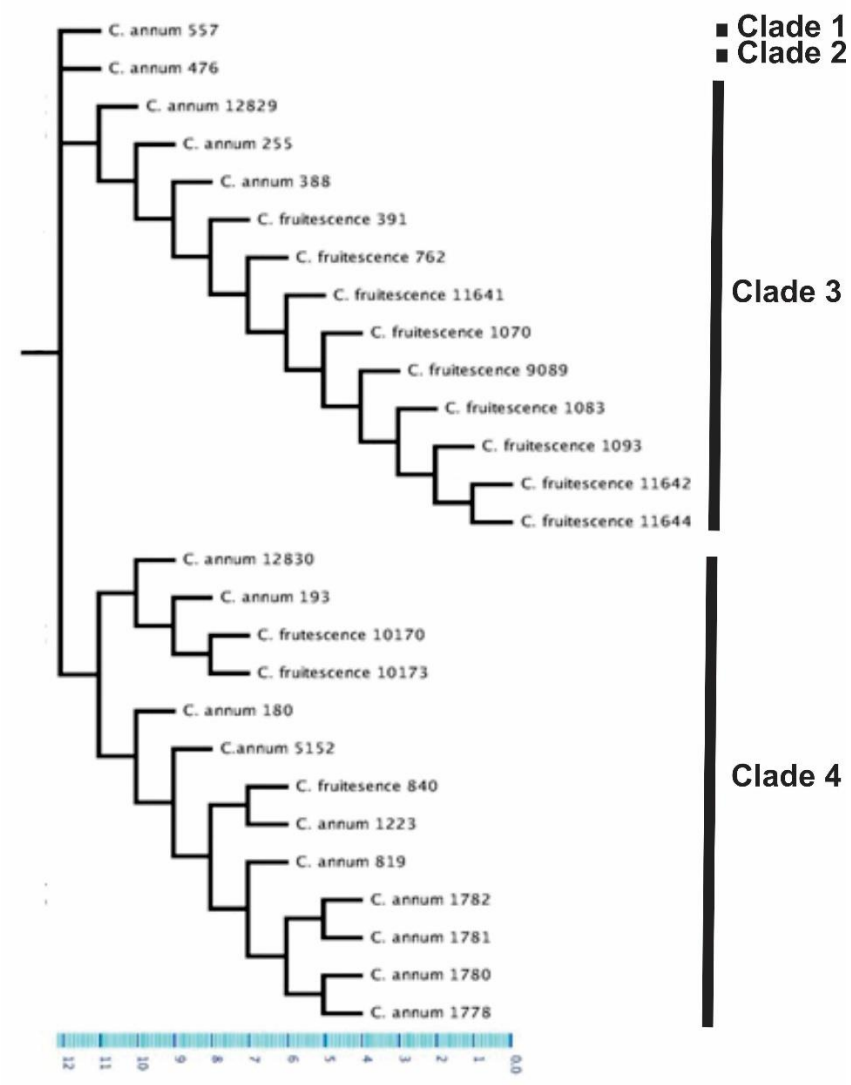

Fig. 01 - Phenogram of twenty-seven (27) Capsicum accessions

Clade number four (04) is further divided into two clades separating one cluster only for *Capsicum annuum* accessions; 180, 5152, 1223, 819, 1782, 1781, 1780, and 1778. As per the previous study

done by analyzing rDNA-ITS similar results were obtained by proving monophyly of the *C. annuum* with a 71% support value by Shiragaki et al., 2020. However, *Capsicum frutescens* accession number 840 may be misclassified, because the corolla color of 840 was greenish, while it is purple in general (Eshbaugh, 2012). The second division of the clade 4 again indicates the *Capsicum annuum* as the common ancestor of the morphologically similar *Capsicum frutescens* accessions; 10170 and 10173. These two accessions had 75% of morphological similarity to the studied qualitative and quantitative characteristics evaluated. The results of the present study are in partial agreement with the previous studies done with the molecular marker analysis (McLeod et al, 1983; Ince et al., 2010; Jeong et al, 2010; Silvar et al, 2016), at the same time we were unable to entirely different between the *C. annuum* and *C. frutescens.* The analysis using molecular markers or DNA barcoding requires many Capsicum species for species identification, and thus takes a lot of effort. On the other hand, phaenogamic presentation of the traits will not cater entirely resolve the phylogenetic relationships. Therefore, phylogenetic analysis using other reliable genetic markers is essential.

4.0 CONCLUSION

The phonogram generated from numerical comparisons of thirty-eight quantitative and qualitative descriptor states is characterized by displaying morphological diversity among Capsicum accessions by separating them into four (04) major clades. *C. annuum* accessions; 180, 5152, 1223, 819, 1782, 1781, 1780, 1778 had monophyletic relationships by nesting into one cluster. Other accession numbers did not have any consistency while placed in the tree firmly. Therefore, the present study does not reveal the morphological phylogeny entirely of the studied Capsicum accessions. Although morphological characterization is recognized as the starting point in the assessment of genetic diversity, morphological markers should be complemented with molecular markers for more advanced genetic diversity study in Capsicum.

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Evaluating the Antibacterial Activities of Selected Plant Extracts in Different Solvents

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Abstract – *Several species of bacteria are responsible for the most food born deteriorate. Among them Bacillus spp. Escherichia coli and Micrococcus are the major causal organisms. Synthetic bactericide is one of most effective controlling method with health hazard. Hence, there is an urgent need for developing a bio safe method to control food borne bacterial pathogens. The aim of the present study was to investigate the Antibacterial effect of Adhathoda vasica - leaves, Azadirachta indica - seeds, Ricinus communis - seeds, Clerodendrum infortunatum-leaves and Pistia stratiotes- leaves via in vitro testing. Plants were extracted in five different solvents; methanol, ethanol, chloroform, petroleum ether and sterilized distilled water. Disk diffusion assay was conducted to evaluate the performances of each plant extract against Bacteria. A wide range of the yields among extracts was observed based on the solvent and the plant material. Ethanol plant extracts were performed best, having highest inhibition zones between 10 - 14 mm including the control with solvent only. Chloroform and Methanol plant extracts were resulted with moderate inhibition zones, while distilled water and petroleum ether were least effective against all bacterial species. The highest inhibition zone of 13.66±1.52 mm observed by Micrococcus applied in ethanolic Ricinus communis plant extract. Adhathoda vasica with ethanol was found most effective against all three tested organisms, where 12.41±2.00 mm in zone of inhibition observed in Escherichia coli cultured in ethanolic extracted Clerodendrum infortunatum while zone of inhibition was observed as 12.00±1.00 mm bacteria growth of Bacillus species. The study revealed that there is a possibility of using these plant extracts on controlling food borne diseases.*

Key words: *antibacterial effects, plant extracts, solvents, zone of inhibition*

1. INTRODUCTION

Life without nature is impossible for human beings. Food, clothes, and shelter are three basic needs of human beings, and health is the most important need, which is provided by the plant kingdom through a healthy food supply (World Health Organization 1992). Public and health are concerned with microbiological safety and quality of food due to the emergence and reemergence of foodborne pathogens across the globe (Odeyemi and Bamidele 2016; Odeyemi and Sani 2016). More than 250 sources of foodborne diseases have been identified worldwide (Scallan et al. 2015). Foodborne diseases are among over 13 zoonoses associated with over 2 billion illnesses worldwide, and more than 2 million deaths are recorded every year because of them in developing countries (Kelly et al. 2014).

Food borne diseases, caused by various bacteria, *bacillus spp. Escherichia coli* and *Microccous spp affect* agricultural food productions. Bacteria and viruses are typically the cause of food borne illnesses. Generally harmful bacteria may be already present in foods when you purchase them. Raw foods including meat, poultry, fish and shellfish, eggs, unpasteurized milk, dairy products and fresh produce often contain bacteria that cause food borne illnesses (Todd, 2014). Traditionally, Organic acid and food preservatives are used to control food borne disease and extend the shelf life of processed food. Antimicrobial agents, including food preservatives and organic acids, have been used to inhibit food borne bacteria in the food industry. Plants, herbs, and spices naturally occurring as antimicrobial compounds can serve as a source for antimicrobial agents against food pathogens (Deans and Ritchie 1987; Janssen et al. 1985).

Extracts containing different classes of phenolic compounds from many plants have recently gained popularity as well as scientific interest for their antibacterial and antifungal activity; Verástegui et al., 2008; Santas et al., 2010, Rauha et al., 2000; Al-Zoreky., 2009). Phenolic compounds are one of the rich sources of biocides and preservatives explored by scientists for a long time as postharvest alternative control (Lattanzio., 2003). The components such as carvacrol, eugenol, and thymol with phenolic structures, were highly active against the plant pathogens. Sri Lanka, with its rich biodiversity, is blessed with many unexplored wild herbaceous species with different capacities and which is possible to incorporate into crop development by means of fertilizers or as pesticides. They are interesting from an ethno-botanical point of view, since a lot of them are used in Sri Lanka as source of drugs in traditional and Ayurveda medicine. Furthermore, they are well known as a rich source of anti-inflammatory, diuretic, antioxidant, antibacterial, and antiviral active substances, with cosmetic as well as medicinal values (Yukawa et al., 1996; Dhiman and Chawla., 2005; Wang., 2006; Wang and Lobstein 2006).

The literature claims very low number of explorations of antimicrobial activity of phenolic extracts obtained from wild species against foodborne pathogens. Therefore, the objective of the present study was to evaluate the *in vitro* antibacterial activity of different solvent extracts of five medicinal plants. Preliminary data were analyzed to study the efficacy of the different solvent extracts of selected plants in preventing the growth of three bacterial species.

2 METHODOLOGY

2.1 Plant Materials

Abandonly available five plant species (*Pistia stratiotes, Adhathoda vasica, Ricinus communis, Clerodendrum infortunatum, Azadirachta indica*) were collected from Low Country Wet Zone in Sri Lanka and classified according to botanical and family names (Table 1). Plant parts were collected as given in the table 1 for extraction. Fresh plant parts were labeled and transported to the laboratory in sealed bags. They were cleaned and washed with distilled water followed by washing with 5% of Sodium hypochlorite (NaOCl) added with few drops of Tween-20 were used as a disinfection.

Table 1: Details about the plants used for the study

Scientific name	Common name	Plant Part used

Pistia stratiotes	Water lettuce	Leaves
Adhathodavasica	Adathoda	Leaves
Ricinus communis	Castor plant	Seeds
Clerodendruminfortunatum	Hill glory bower	Leaves
Azadirachta indica	Margosa	Seeds

2.2 Preparation of plant Crude Extracts

Analytical grade solvents; methanol, petroleum ether, ethanol, chloroform and the sterilized distilled water were used as extraction solvents. Plant tissues were homogenized by following the method described by Gurjar et al., in 2012 with slight modifications. Plant materials were grounded by using sterile mortar and pestle by adding sample: solvent as 1:10. Finely ground plant materials were subjected to shaking at 100 rpm for 24 hours at room temperature. Extracts were filtered three times with What-man 42 filter papers, by adding relevant solvent on each time followed by centrifugation at 4000 rpm for 20 minutes. Filtrate was concentrated through rotary evaporator until a sticky dark green crude extract was obtained at 700 ppm pressure and 50 °C for methanol, ethanol, petroleum ether and chloroform and at 0°C for distilled water. The crude extracts were stored in an airtight container at 4◦C until further use.

2.3 Preparation of the bacterial cultures

The pure bacterial Cultures of *Escherichia coli, Micrococus species* and Basilus *species* were collected from The Department of Botany, Faculty of Natural science, The Open University of Sri Lanka, Nawala, Nugegoda. The bacteria cultures were sub cultured from Mueller-Hinton Agar (MHA HiMedia) in Disk diffusion assay**.** Tryptic Soy Broth (TSB) media was prepared by following the manufacturer description for liquid bacterial inoculum cultures aiming rapid growth of bacteria cells.

2.4 Antibacterial assay

Antibacterial activates were performed through Disc Diffusion method by following the method explained by Mahesh and Satish (2008) with slight modifications. The pure bacterial Cultures of *Escherichia coli, Micrococcus species* and *Bacillus* species were sub cultured by streaking and inoculating into prepared Muller Hinton Agar (MHA) medium and incubated at 30 °C overnight for all bacterial species. After overnight incubation, well grown isolated colonies were streaked using inoculating wire by dipping in to aperture tube containing 2 ml of Tryptic Soy Broth (MHB). The broth culture was incubated at 35±2°C for 18 hours, centrifuged at 10,000 rpm for 10 minutes. Then the pellet was suspended in double normal saline (0.90% NaCl) to acquire the concentration of 10^6 CFU/ml of the cell density which was standardized by back calculation by plating 10^2 and 10^1CFU/ml. A bacteria cultures, which has been adjusted to 0.5 McFarland standard, were used to lawn Muller Hinton agar plates evenly using a sterile spreader. Then plates were dried for 15 minutes and then used for the sensitivity test. After solidification the filter paper discs with 5.5 mm in diameter sterilized by Autoclaving at 121°C for 20 minutes in

1.05 Kg/cm^3 finally oven dried at 60 ^{o}C for overnight were pressed down to ensure complete contact with the agar surface and distributed evenly so that they were no closer than 24 mm from each other, center to center. Then impregnated with the 10 µl (100 µg/ml) extracts. The plates were inverted and placed in an incubator setting the temperature to 35°C within 15 minutes after the discs were applied. Plates were then incubated for 24 h at 37°C as described by the Salie et al. (1996) and Baris et al. (2006). After incubation each plate was examined. Diameter of zone of inhibition was measured to the nearest whole millimeter at the point wherein there is a prominent reduction of 80% growth. Solvents were used as negative controls. All assays were carried out in triplicate.

2.5 Data and statistical analysis

Microsoft excel software (version 13) was used for basic descriptive statistical analysis. Linear growths (LG) for antibacterial activities were calculated through measuring the inhibition zones diameter in millimeters. Antibacterial activities were measured by formula described by Mahmood *et al*., 2012. Data was analyzed by one way ANOVA by using GraphPad Prism 6.0 (Solvusoft Corporation, Las Vegas, NV, USA**)** at 95% confident level ($P < 0.05$).

3.0 RESULTS AND DISCUSSION

Escherichia coli, Micrococcus and *Bacillus species* were very sensitive to *Adhathoda vasica* - leaves, *Azadirachta indica*- seeds, *Ricinus communis* - seeds, *Clerodendrum infortunatum*- leaves and *Pistia stratiotes*- leaves as bacterial growth of this bacteria was inhibited or reduced when the growth media was amended with plant extracts. The result of the *in vitro* screening tested against *Escherichia coli, Micrococcus* and *Bacillus species* revealed that there was a significant difference (*p<0.005) in antibacterial effect among treatments when used different solvents; methanol, ethanol, chloroform, petroleum ether and sterilized distilled water. The inhibitory activity of plant extracts may be due to direct toxic effects exerted by the pathogens (Choudhury et al. 2018).

(a) (b) (c)

Plate 1: Inhibiting the growth of (a) *Escherichia coli, (b) Bacillus* species (c) *Micrococus* species by five different plant extracts

There was a significant difference among the antifungal effects among methanol, ethanol, chloroform, petroleum ether and sterilized distilled water solvents with five different plant extracts when inhibiting the growth of *Escherichia coli, Micrococcus* and *Bacillus species* as per the figures 01, 02 and 03. The high inhibition zones of 13.66±1.52 mm was observed against *Micrococcus* by ethanolic *Ricinus communis* extraction. *Adhathoda vasica* extracted in ethanol was found most effective against all three tested bacteria, where 12.41±2.00 mm zone of inhibition was measured against *Escherichia coli* and *Clerodendrum infortunatum* ethanolic extraction subpress the growth of *Bacillus* species by 12.00±1.00 mm. Ethanol extract showed significant effect on *in-vitro* inhibition against all the pathogens respectively. However, when compared with other plant extracts *Ricinus communis* had significantly higher inhibition for *Micrococcus.*

In the present study, the five plants extract screened with five solvents showed different effects on *Basillus* species. Ethanol extract of *Clerodendrum infortunatum* showed highest inhibition of the growth of *Basillus* species by allowing to measure inhibition zone of 12.00 mm. Ethanolic *Azadirachta india* and *Adhathoda vasica* inhibited the growth of *Basillus* by acquiring 11 mm and 10.31 mm zone of inhibitions respectively. Ethanolic *Ricinus communis* extract resulted with 7.8 mm inhibition in *Basillus* species. Only ethanol solvent had slightly high zone of inhibition (figure 01). According to the figure 01, *Clerodendrum infortunatum* showed higher inhibition of *Basillus species* than other plant extracts.

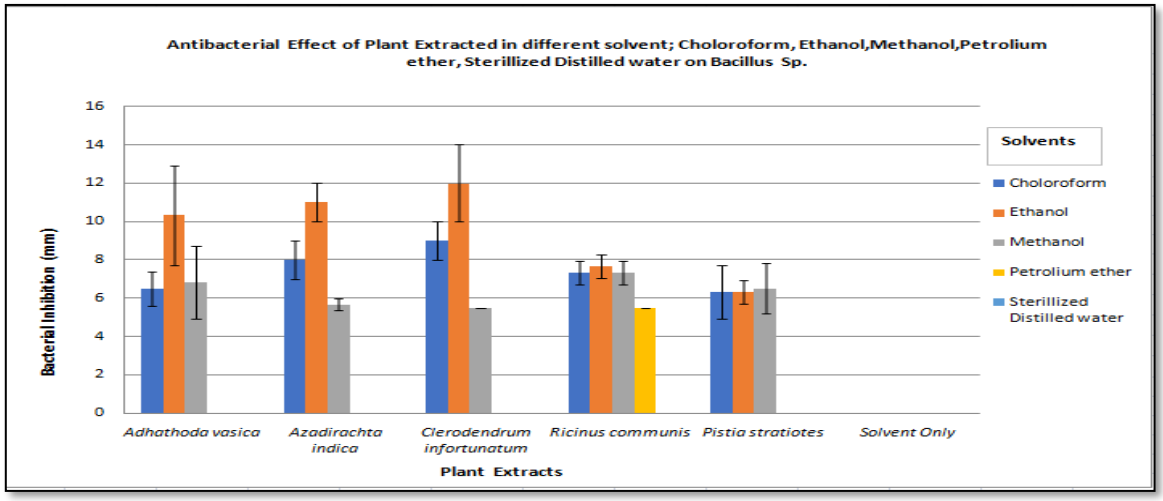

Figure 01: Total Effect of leaf and seed extracted in different solvents; Chloroform, Ethanol, Methanol, Petroleum ether and Sterilized Distilled water, on inhibition of *Bacillus species*

Ethanolic *Adhatoda vasica* extraction resulted with 12.41 mm inhibition of *Escherichia coli* as well as Ethanolic, *Clerodendrum infortunatum* leaves, *Azadirachta indica* seeds and *Pistia stratiotes*- leaves extracts were capable gain 12 mm, 10.88 mm, 10.88 mm, inhibitory zones respectively for the growth of *Escherichia coli* (figure 02). Ethanol also gave a highest inhibition comparatively with than other four solvents. This has to be further studied to clarify the effects of the ethanol on growth inhibition on *Escherichia coli*. In addition, chloroform as well as methanol were shown higher inhibition zones in control where required further studies for clarification. Ethanolic *Ricinus communis* extracted showed significantly higher growth inhibition of *Micrococcus* than other two bacteria, beyond that, ethanolic extracted *Azadirachta indica* and Methanolic extracted *Pistia stratiotes* gave

comparatively high growth inhibition zones of the *Micrococcus* were measured as 11.51 mm and 11.44 mm.

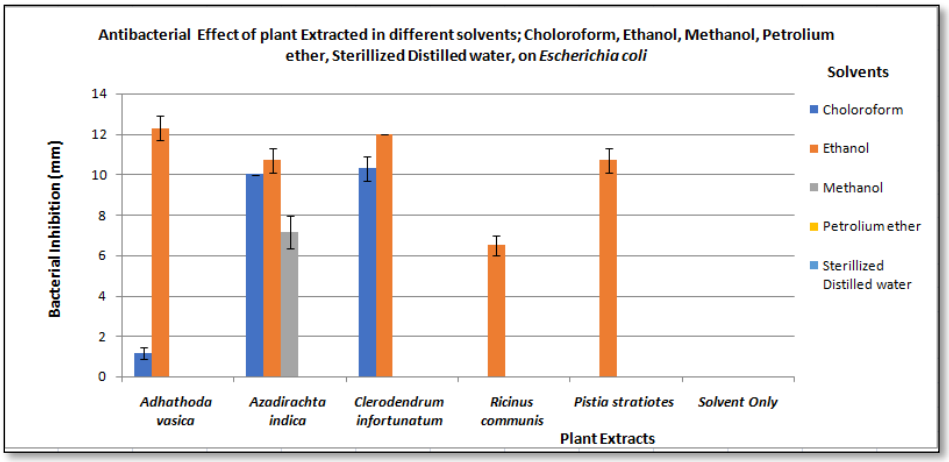

Figure 02: Total Effect of leaf and seed extracted in different solvents; Chloroform, Ethanol, Methanol, Petroleum ether and Sterilized Distilled water, on inhibition of *Escherichia coli*

As per the findings of Hashem Rahmati et al. in *2015,* the crude extract of *Ricinus communis* seeds was found to be contained phytochemical compounds of anthocyanin, sterol, tannins and essential oils. The phenols, tannins, alkaloids, anthraquinones, saponins, flavanoids, aminoacids and reducing sugars are present in the leaves of *Adhatoda vasica (*Karthikeyan et al. 2009). The numbers of *Clerodendrum* species were documented in ancient texts for their antimicrobial action (Neeta and Tejas 2007). Tannins are biologically active against *E. coli*, *S. aureus*, *S.paratyphi* and *C. albicans* as per the study done by Harborne et al. in 1993. Therefore, that is the reason these three plant extracts highly influence to minimize the growth of *Basillus* species, *Escherichia coli* and *Micrococcus.*

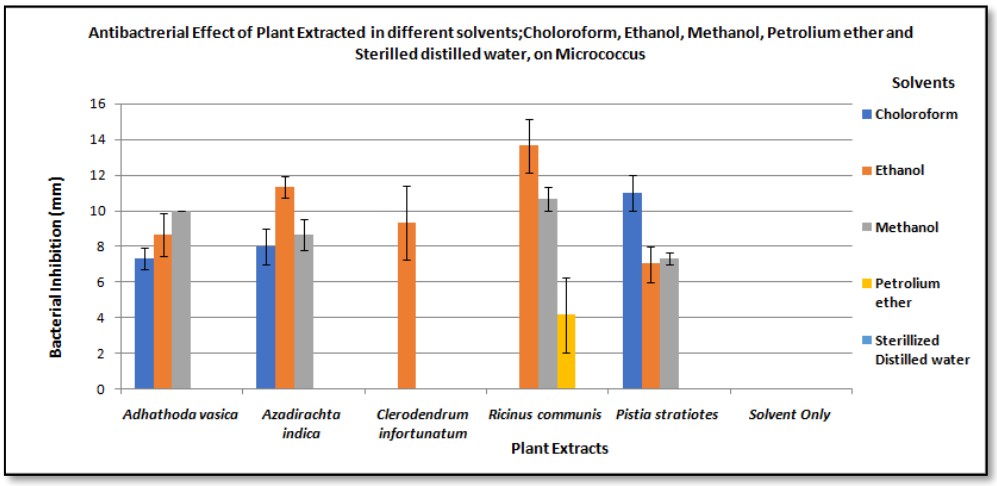

Figure 03: Total Effect of leaf and seed extracted in different solvents; Chloroform, Ethanol, Methanol, Petroleum ether and Sterilized Distilled water, on inhibition of *Micrococcus*

Moreover, as per the figures 01, 02 and 03, with respect to the solvents used for extraction in the present study, ethanol performed best, having shown higher inhibition zone in

between 06 - 14 mm range including the plant extract with ethanol towards all three bacteria studied. Cowan, (1999) reported that ethanol and methanol can extract more active components such as alkaloids, tannins, flavonol, terpinoids, and flavones. Based on the literature and the food regulations, ethanol is known as a good solvent for certain selected food products (Alzeer and Hadeed, 2016). The antimicrobial activities of *Ricinus communis* were good against pathogenic bacterial strains *Streptococcus progenies, Staphylococcus aureus, Klebsiella pneumonia,* and *Escherichia coli* (Jena *et al*., 2012). In this study *Ricinus communis* with ethanol and methanol extracts were observed significant effect against *Micrococcus* which was high inhibiting effect of 13.66±1.52 mm diameter zone of inhibition. Uwimbabazi Francine et al., (2015) reported that Ethanol leaf extract showed higher inhibition effect than aqueous and other extract study on enable trace and witness of Neem and how it is effective on some pathogens causing diseases such as *Staphylococcus aureus* and *Escherichia coli* as experienced in the present study. The numbers of *Clerodendrum species* were documented in ancient texts for their antimicrobial action (Neeta and Tejas 2007). *Clerodendrum infortunatum* leaves extract showed effective results than root and stem extracts. The ethanol an ethyl acetate extracts were possessed a wide spectrum of antibacterial action against Gram-negative and Gram-positive bacteria (Taluar, 2014). Therefore, this study showed the highest zone of inhibition in ethanolic *Ricinus communis* – seed extracts against to bacteria *Micrococcus.*

4.0 CONCLUSION

In the present study, the natural product extracts were identified as the inhibiting agents of Escherichia *coli, Micrococcus* and *Bacillus* species with high potency. The most effective plant extracts against the three species are *Ricinus communis*, *Clerodendrum infortunatum* and *Azadirachta indica.* The preset study also confirms the *in-vitro* synergistic effect of ethanolic *Ricinus communis- seeds against the Micrococcus* resulted highest zone of inhibition. Isolation of the active compounds from ethanolic extracts could lead to improved antibacterial use in agriculture to preserve food crops as well as in the pharmaceutical industry for treatment of various bacterial diseases. Moreover, the results of the study will form the base of selection of plant species for further investigation with the potential discovery of new natural bioactive compounds.

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