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SITE-SPECIFIC MACRONUTRIENT MANAGEMENT FOR CAULIFLOWER (*Brassica oleracea* L.) IN BATAPOLA SRI LANKA

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ABSTRACT

A blanket fertilizer was recommended by the Department of Agriculture (DOA) for all species of cabbage. However, the fertilizer application is needed to be site-specific. Hence a field experiment was conducted at Ambalangoda during the Maha season in 2019 with the objective of evaluating the effect of Calcium (Ca), Magnesium (Mg) and Boron (B) on the yield and the Quality of cauliflower. Batapola Ambalangoda soil was analyzed for available nutrients and identified the most deficit macronutrients as Ca, B and Mg. This was laid out in a Randomized Complete Block Design with four replicates. One plot size was 1m × 2m and each plot had ten plants. Five treatments were taken as NPK (DOA recommendation) (T1), NPK + Ca + Mg (T2), NPK + Ca + Mg + B (T3), NPK + Ca (T4), NPK + Mg (T5). Incorporating magnesium, calcium and boron with the DOA recommendation resulted in larger cauliflower curds under field conditions, contributing to a 44.2 % increase in curd yield. The additional four days of shelf life were observed in the curds of T3.

Keywords: Blanket fertilizer, Cauliflower, Micronutrients, Site-specific nutrient management

INTRODUCTION

Cauliflower (*Brassica oleracea* L.) is a cruciferous crop grown for its white curds, which are edible. This crop grows in a cool, wet climate and is sensitive to extreme temperatures (Din et al., 2007). Cauliflower's poor yield is caused due to many factors; among them, imbalanced and low availability of specific nutrients is the major limitation (Bairwa et al., 2021). Hence the soil was collected from the experimental site in Batapola, Ambalangoda and available nutrients were analyzed.SSNM(site-specific nutrient management) is a method of providing nutrients to plants that best match their natural spatial and temporal nutritional requirements (Verma et al ., 2020). Site-specific management, in association with 4R Nutrient Stewardship, provides perfect crop management systems and utilizes the right source, rate, time, and location of nutrients to be applied (Mascarenhas., 2014). As a result of site-specific nutrient management, it will Increase fertilizer use efficiency and reduce nutrient losses. A site-specific nutrient estimate is developed based on preliminary soil analysis and sorption tests (Ningawale et al., 2016). Application of the micronutrient as calcium, magnesium and boron along with the recommended doses of macronutrients such as nitrogen, phosphorus, and potassium boast up yield component of cauliflower (Kumar et al., 2012). According to (Tiwari., 2007), the following are the key characteristics of SSNM: Based on soil testing, primary and secondary nutrients are applied to specific sites making the best use of existing nutrients such as those found in soil residues parts and manures whereas SSNM also offers directions for selecting the most cost-effective nutritional combinations. Three essential phases in site-specific nutrient management include assessing soil and crop variability, controlling the variability, and evaluating it (Choudhary et al., 2016). We can moderate variability and conduct precision farming by using the current technologies to assess variability and provide site-specific agronomic solutions. This site-specific nutrient management includes the identification of nutrient deficiency in that specific site and the formulation of fertilizers to compensate for that deficit. Balanced fertilization is required to maintain optimum soil health, including the application of all necessary plant nutrients in the right amount and form (Singh H et al., 2010). As a result, this study aimed to develop a site-specific secondary macronutrients suggestion for cauliflower in Batapola and assess the effectiveness of the fertilizer. Calcium (Ca), Boron (B) and Magnesium (Mg) were the secondary macronutrients that were deficient in the experimental site and therefore, for the recommendation only deficit quantity was added.

METHODOLOGY

This field experiment included five treatments with slightly varying magnesium, boron and calcium combinations, and it was compared to the current Department of Agriculture (DOA) standard. The field experiment was conducted from September 2019 to December 2019 at the Batapola, Ambalangoda during the Maha season. The experimental site belonged to the Red Yellow Podzolic soil group in the Low Country Wet Zone of Sri Lanka. The soil samples were tested in two replicates for available nutrients using a suggested preliminary extraction process by Portch and Hunter (2002) at a depth of one meter. The calcium and magnesium were detected by an atomic absorption spectrophotometer (Buck Scientific model 210 VGP). The fertilizer combinations for the treatments were created using the technique provided by Portch and Hunter, based on the nutrient analysis of the original soil sample and data from the fixation research (Portch and Hunter.,2002). Five treatments were tested under field conditions. The treatments (Table 1) were taken as DOA recommendation (Control) (T1), NPK with Ca and Mg (T2), NPK with Ca, Mg and B (T3), and NPK with only Ca (T4), NPK with only Mg (T5). The Experiment was designed under a Randomised Complete Block Design (RCBD) with four replicates. Urea 110 kg/ha and Triple Super Phosphate (TSP) 275 kg/ha and Muriate of Potash (MOP) 75 kg/ha were applied as basal dressing, whereas 110 kg/ha of urea was applied as top dressing 1 and as top dressing 2, urea 110 kg/ha and MOP 75 kg/ha was applied. Seedlings of cauliflower variety 'Fuji' was transplanted in the field according to the DOA recommended spacing of 50 cm x 40 cm. Light traps were fixed to control plant hopper attacks and pesticides were used to control insects and diseases and white polythene was laid around the field to protect the plants from wild boar. Department of Agriculture recommendation was identified as T1 (Table 1). Urea, triple superphosphate, muriate of potash, calcium nitrate, magnesium sulphate, and boric acid were used to supply nitrogen, phosphorus, potassium, calcium, magnesium and boron respectively.

Plant characteristics such as plant height and total leaf area, number of days to 50% flowering, and terminal yield /ha were measured and analyzed during the preharvest period for each treatment. Harvesting was done when the curd reached commercial maturity, which was when the curd was well-formed, firm, and compact with a very close texture and white or cream colour, which was the optimal harvesting stage for the best curd quality (Kirthisinghe, 2006). The curd's colour and compactness were visually appraised, and measurements were taken using a visual quality rating (VQR) scale established specifically for this study based on the Sri Lankan market value for cauliflower curd. Keeping Quality (shelf life) as a post-harvest quality evaluation was performed using the curds collected, with four curds from each fertilizer treatment randomly selected and stored separately. Two curds were maintained in the open air at room temperature 28+2 ºC among these four curds whereas the other two curds were kept in 150 gauge low-density polythene bags at room temperature 28+2 ºC with a relative humidity of 93+3 %. As a quality parameter, the storage life was calculated by counting the number of days it took to show the first signs of rotting, decaying, or colour change. The data were analyzed using the Statistical Analytical Software (SAS, 2005) package. For normally distributed data, ANOVA with the Duncan Multiple Range Test (DMRT) was used.

RESULTS AND DISCUSSION

The preliminary soil study revealed that the CEC is 5.3 cmolkg^{-1} in the experimental location of Batapola, Ambalangoda and that calcium and magnesium were deficient, with the available calcium content of 740 mg/kg and magnesium content of 193 mg/kg whereas boron was not detected. The soil had a pH of 6.3, and available nitrogen, phosphorus, and potassium at 0-15 cm depth were 26, 11, and 112 mg/kg, respectively, as determined according to AOAC 2006.03. (2009) and the official analysis method (Hall William., 2006).

Curd yield

The average curd yield for the control treatment current study's DOA recommendation was 5.4 t/ha. At Ambalangoda soil, NPK with Ca, Mg and B treatment produced a better yield of 7.8 t/ha. T3 got a 44.2 percent yield increase compared to the control (Table 4). The results revealed that NPK with Ca , Mg improved production by 35.1 percent and NPK in combination with Ca raised yield by 33.3 percent whereas NPK with Mg increases yields by 25.9 percent (Table 4).

Mean plant height of the cauliflower

The plots treated with NPK with Ca, Mg and B gave a significantly higher ($p<0.05$) mean plant height (10.4 cm). The minimum mean height (8.6 cm) was attained in T1, whereas the T2 recorded an average height of 9.3 cm (Table 3).

Total leaf area of the cauliflower plant

The total leaf area per plant (cm^2) was used as an indicator of plant vegetative development and as a source of synthetic material for curd growth in connection to fertilizer treatments. The leaf area at 6 WAP (weeks after planting) differed significantly ($p<0.05$) among fertilizer treatments (Table 2). T3 had the highest total leaf area (2898.9 cm^2) whereas the control had the lowest total leaf area (2554.1 cm^2) at 6 WAP.

Table 1. Treatment mixtures for the field experiment at Batapola, Ambalangoda.

	Treatment	N	P_2O_5	K_2O	Ca	Mg	B
		kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	Kgha-1
T1	NPK (Recommended)	150	125	90	0	0	0
T2	NPK + Ca + Mg	150	125	90	400	100	0
T3	NPK + Ca + Mg + B	150	125	90	400	100	10
T4	NPK + Ca	150	125	90	400	0	0
T5	NPK + Mg	150	125	90	0	100	0

Table 2. The total leaf area per plant (cm^2) and shelf life of curd (days).

	Treatment	Total leaf area at 6WAP (cm^2)	Shelf life of curd (Days) Opened	Polythene wrapped
T1	NPK (Recommended)	2554.1 ^{d}	10^{bc}	16^{bc}
T2	NPK + Ca + Mg	2570.0 ^{cb}	12^{b}	21^{b}
T3	NPK + Ca + Mg + B	2898.9 ^{a}	14^{a}	24^{a}
T4	NPK + Ca	2698.2 ^{c}	8^{c}	14^{c}
T5	NPK + Mg	2740.3^{b}	11^{b}	18^{b}

*Values within a column followed by a common letter are not significantly different at P=0.05, according to DMRT.

Table 3. The mean plant height (cm), number of days to 50% flowering (days) and average diameter of the curd (cm).

	Treatment	Mean plant height (cm)	Number of days to 50% flowering (days)	Average diameter of the curd (cm)
T1	NPK (Recommended)	8.6 ^{bc}	41 ^{cb}	8.9 ^{bc}
T2	NPK + Ca + Mg	9.3 ^{d}	39 ^{b}	11.4 ^{d}
T3	NPK + Ca + Mg + B	10. 4 ^{a}	35 ^{a}	12.1 ^{a}
T4	NPK + Ca	8.2 ^{bc}	43 ^{c}	9..2 ^{bc}
T5	NPK + Mg	9.1 ^{b}	38 ^{b}	11.3^{b}

*Values within a column followed by a common letter are not significantly different at P=0.05, according to DMRT.

Table 4. The average final yield with yield gain percentage at the experimental site.

	Treatment	Final Yield (t/ha)	Yield gain %
T1	NPK (Recommended)	5.4 ^{c}	0
T2	NPK + Ca + Mg	7.3 ^{b}	+ 35.1 %
T3	NPK + + Ca + Mg + B	7.8 ^{a}	+ 44.2 %
T4	NPK + Ca	7.2 ^{b}	+33.3 %
T5	NPK + Mg	6.8 ^{d}	+25.9 %

*Values within a column followed by a common letter are not significantly different at P=0.05, according to DMRT.

Number of days to 50% flowering

The variety 'Fuji' is an early flowering type variety. It took 36-45 days to show 50% flowering and 22–25 days from the flower initiation to get the curd matured for harvesting. Significant differences in the number of days to 50% flowering were observed among treatments ($p<0.05$) where the lowest number of days to 50% flowering was in T3 (35 days). The DOA recommended treatment showed the highest number of days (41 days) to 50% flowering (Table 3).

Curd shelf life

Significant differences ($p<0.05$) were found between fertilizer treatments in the shelf life of cauliflower curd during storage under two various storage settings. Curds of T3 had the longest storage life (24 days) when kept at a high relative humidity in polythene bags. In both environmental conditions, the fertilizer treatment T4 had the shortest storage life. T3 in the open air at room temperature shelf life was (8 days) whereas the curds were kept in polythene bags at room temperature shelf life was (14 days) (Table 2). When the storage life of curds kept in polythene bags with a relative humidity of 93+3 percent was compared to the storage life of curds stored in other treatments, the curds stored in polythene bags with a relative humidity of 93+3 percent had the longest storage life. When compared to alternative storage settings, curds stored in polythene bags had the longest storage life, resulting in low oxygen levels and high carbon dioxide levels. T3 treated curds stored in polythene bags showed an additional 8 days of storage when compared to the curds from the T1 (DOA recommendation) stored under respective conditions.

CONCLUSIONS

Results indicated that the yield, plant height and leaf area were higher in the NPK fertilizer mixture with Ca, Mg and B (T3) which were deficient in the experimental location. Post-harvest keeping quality is also high in the T3 compared to the control (T2). The incorporation of magnesium, calcium and boron with the DOA recommendation formed larger cauliflower curds under field conditions, contributing to a 44.2 % increase in curd yield. Adjustment of micronutrients with the DOA recommendation according to the specific site is a significant effect on the yield of cauliflower. The additional four days of shelf life observed in the curds obtained from plots treated with T3 would provide a considerable advantage, over the other treatments. This site-specific nutrient management includes the identification of nutrient deficiency in the specific site and the formulation of fertilizers to compensate for that deficit. It is the best method to attain a sustainable high yield without adversely damaging the environment, especially the soil.

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A REVIEW ON *Calotropis gigantea* (WARA): A MIRACLE PLANT

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ABSTRACT

Calotropis gigantea (L.) R.Br. (Asclepiadaceae) is a perennial herb used as a traditional medicine. This is an important plant however, presently under the threat of extinction. While the entire plant is used for skin diseases, powdered root bark able to cure elephantiasis, dysentery and leprosy. The stem bark is important for gastrointestinal and respiratory track diseases including dysentery, spleen complaints, ringworm and pneumonia. The toothache, caries, leprosy, stings, syphilis, rheumatism and tumors were treated using latex of the plant. Powdered flowers have therapeutic value for coughs, colds, and asthma. The leaf extraction has potential to heal burns and relief headaches, rheumatic pains. Mosquito repellent efficacy of flower extracts was already published. In addition, because of the massive forest destruction, it has been included in the Red Data Book of IUCN. This paper will discuss the significance of this species and the medicinal value to convince the importance of conservation by reintroducing regenerated plants into their natural habitats

Keywords: Calotropis gigantean, Traditional Medicine, Red data book, Wara

INTRODUCTION

Calotropis gigantean 'Wara', is a drought-resistant and salt-tolerant plant belongs to the Apocynaceae family (Orwa *et al*., 2009) that usually referred to as "Giant milkweed" and locally referred to as "*wara"* in Sri Lanka.It has several names in various nations and even in the same country it is called by different names. As an example; Arka in India, Remiga in Malaysia, Bidhuri in Indonesia, Kapal-kapal in Philippines, Po thuean in Thailand, B[oot]ng b[oot]ng in Vietnam, Faux arbre de soie in French. However, in English it is called as English Crown flower, giant Indian milkweed (Kumar *et al.*, 2013). *Calotropis* is native to Sri Lanka, Thailand, China, Cambodia, India, Malay Islands, Philippines, and Indonesia. Moreover, it is distributed within; Myanmar, Niger, Nigeria, Nepal, Oman, Pakistan, Senegal, Sudan and Saudi Arabia (Poonam & Punia, 2013).

The purpose of this review paper is to identify and introduce the properties of *C.gigantean*, as well as to summarize the health benefits in a broad sense. In some countries, this species has already been designated as an endangered species, and this paper will discuss the importance of the plant and also go through some uses of *C.gigantean*. Therefore this article is intended to be useful for both scientific and lay audiences.

Botanical Characters

The family of Apocynaceae mainly comprises of latex bearing plants with more than 280 genera and 2,000 species (Mushir *et al*., 2016) that are fundamentally conveyed to the tropical and subtropical Africa and Asia. Apocynaceae are the placed in the Gentianalels order under the Asteridae subclass (Rahman & Wilcock, 1991).

Kindom: Plante
Order: Gentianalels
Family:Apocynaceae
Subfamily:Asclepiadaceae
Genus: *Calotropis*
Species: *Calotropis gigantea* (L.) W.T.Aiton

Morphological Characters

This grows up to 4-10 m like a large shrub or small tree. It has erect stems with 20 cm diameter consist with pale gray color barks and young woolly hairy shoots. Latex can be seen in all part of the plant (Plate: 01-A).

Thick leaves with opposite: decussate leaf arrangement but simple, not present petiole, Stipules are absent (Plate:01-B) Blade is widely ovate to oblong-obovate, 9.5-20 cm× 6-12.5 cm, apex is almost as acute type , and margins can be categorize under 'entire type' (Plate:01-C) Under the leaf, it's short-hairy (Gaur *et al.,* 2013, Singh *et al.,* 2014).

Axillary type inflorescence and umbellate - corymbose cyme up to 12.5 cm in diameter, 6-12 cm long peduncle and stout secondary branches up to 2 cm long. Bisexual Flowers with white, cream, pale pink or purple in color; the pedicel is 2.5 - 4 cm long. Thickly hairs can be seen.

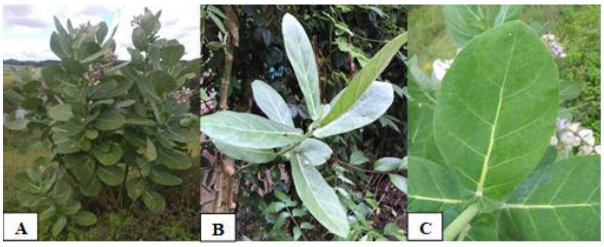

Plate 01: Morphological features of plant and leaf

Plate 02: Morphological features of Inflorescence and flower

Widely ovate calyx lobes and 4-6 mm 2-3 mm ; diameter of corolla is 2.5-4 cm. triangular shape broad lobes and 10-15 mm ×5-8 mm; 5 narrow corona; fleshy scales; laterally compressed, 6-11 mm in long but shorter than the stamina column;forming an upturned horn with two rounded auricles on either side, cream or sometimes lilac to purple in color, superior ovary , two celled, gynostegium up to 1 cm long, stigma head is star in shaped (Plate 02). (Gaur *et al.*, 2013, Singh *et al.*, 2014).

Plate 03: Morphological features of fruit

Insects like monarch, butterflies pollinate flowers. Progeny with genetic differences from their parents (chromosome number 2n = 22). (Payal *et al*., 2015). Each follicle is ovoid and a fruit is a couple of follicles; boat-shaped; inflated; simplistic; fleshy, and seeded. Seeds are 6.5-10 cm × 3-5 cm. ovoid shaped seeds, 5-6 mm in long, small, flat, and have a coma on one end (Plate 03) (Gaur *et al.*, 2013, Singh *et al.*, 2014).

Ecology of plant

Calotropis gigantea thrive in tropical and sub-tropical countries and grows at an altitude of up to 900 meters. It grows well with 300-400 mm downpour fall and mainly in sandy soils with great drainage. However, it can tolerate a variety of soil conditions including poor soils, especially where native grasses have been overgrazed. (Kumar *et al.*, 2013).Moreover, it will not thrive in wet soils without adequate drainage, as roots will affect to the root rot disease. *Calotropis gigantea*, also known as giant milk weed, is a type of weed and Full sun is ideal for growing weed. It blooms through the year and drought is not a limiting factor (Gaur *et al*., 2013). Sri Lanka is an island surrounded by sea water. *Calatropis gigantea* is an ideal plant for beach side cultivation since this is a sun loving plant and having high salt tolerant potential

Propagation

Because the seeds are freely released into the air, regeneration occurs naturally and is very common. Conventionally *C. gigantea* propagated through seed or stem cuttings. Vegetative propagation of stem and root cuttings can be used o produce superior genotypes on a large scale (Kumar *et al.*, 2013). However, then seeds are distributed by water and wind (Gaur *et al*. 2013). Once established it requires only minimal care and do not necessarily require any specific cultivation techniques.

Medicinal properties

"Wara" has potential as a traditional medicine plant with a long history and can be used for many diseases. Root and leaf extracts were showed their therapeutic activities against cancers, syphilis, leprosy, abdominal tumors, and tuberculosis and heal the skin diseases, piles, wounds and insect bites. Roots (highly poisonous) are applied as an acute treatment to snakebites and specially, root bark is used as a treatment for dysentery, purgative, diaphoretic, emetic. Flowers are used for asthma (Motaleb *et al.*, 2011, Verma, 2014). White flower of *C. gigantea* is used to treat diabetes in the past (Manivannan *et al.*, 2017). Cold-related symptoms such as dropsy, rheumatism, and chest pain are treated with a poultice of warmed leaves. The twigs

are used for the preparation of diuretics, stomach tonic, and anti-diarrhoetics and for treatment for the asthma. Also used to treat colic, headaches, lice, toothaches, ulcers, and swellings. (Motaleb *et al.*, 2011, Verma, 2014).

According to some researches, leaf extracts has a large number of bioactive secondary molecules such as alkaloids; tannins; saponin; flavonoids; and glycoside. The presence of these molecules indicated that it might be useful for medicinal purposes (Singh *et al.*, 2014). Moreover, *C. gigantea* leaves have traditionally been treated for stomach ulcers; syphilis; leprosy; types of skin diseases; insect bites; and diseases of elephants, according to Habib & Karim, (2009).

Latex has a strong purgative and caustic effect. For guinea worm blisters, ringworm, and scorpion stings, this is an effective treatment (Mann, 1997). Cholera and indigestion are also treated with the leaf and root –bark (Misra *et al.*, 1993). In India, this plant is used to treat skin conditions such as hypertrophic scars. Ash which has made by using gun powder from *C. gigantea* latex is used in treating dizziness, tooth aches, baldness, hair fall, intermittent fevers, rheumatoid/joints swellings and paralysis. Commercial products as eye tonic, pungent latex extracted from the leaf and flowers of *C. procera* is used (Vohra, 2004).

Some studies proven wound healing activity using the *Calotropis gigantia* latex and incision wound model, while latex has reported significant wound healing activity comparable to the standard FSC (Framycetin sulfate cream) for wounds (Nalwaya *et al.*, 2009).According to Saratha *et al.*, (2011), wild plant latex of *C.gigantea* contains lupeol which is a strong bioactive and widely used as an anti-inflammatory agent.

According to some research, the compounds found in the ethanol extracts of *C. gigantea* has antimicrobial, analgesic, wound healing, cytotoxic, anti-diarrheal, insecticidal, anti - oxidant, anti-inflammatory, anti-carcinogenic, anti-nociceptive properties, as well as hepato-protective properties. Therefore *C. gigantea* is an excellent antimicrobial agent. These findings will undoubtedly have a significant impact on drug and therapeutic research (Pattnaik *et al.*, 2016, Bairagi *et at.*, 2018).

In siddha medicine *C. gigantea* leaves utilized for various treatments. Venomous snake bites, periodic fever diseases, vatha diseases, intestinal worms, and ulcers are all can be treated with this plant. *C. gigantea* roots can be crushed and rubbed firmly on the snake bite area. Dental problems, rodent bites, swelling conditions, gonococcal type arthritis, and other rheumatic complaints are all treated with this plant's latex. Bronchial type asthma can be treated with flowers of this plant (Kumar *et al.*, 2011).

Mosquito repellent activity

C. gigantea flower extracts had a large repellent efficacy against to the *C. quinquefasciatus* vector. Furthermore, *C. gigantea* flower extract can be used as an alternative to traditional insecticides for mosquito control. Moreover, toxicological tests of flower extracts have shown that it is not irritate human skin and that it is safe to use. In the preparation of mosquito repellents, t *C. gigantea* flower can be used alone or in combination with other mosquito repellent plants. Besides, it has a potential to control mosquito breeding in an integrated disease vector. This is a safe mosquito repellent that is both user-friendly and cost-effective (Dhivya *et al.*, 2013).

Industrial uses

Fibers of *Calotropis gigantea* use as a raw material for make carpets, ropes, fishing nets, and sewing thread with the high durability. Seed floss is good for stuffing purposes. The fishing-nets and twine are used Stem-bark yield fiber. The inner bark fiber is crucial for the fabric. (Payal *et al*., 2015). 'Nari' leather is prepared by removing hair from goat skin by fermented mixture of *calotropis* and salt, and of sheep skins to make leather that is commonly used for bookbinding. (Singh *et al*, 1996). In addition, *C. gigantea* is marketed as a houseplant and garden plant in different countries due to its ornamental potential as a butterfly attractor (Al Sulaibi *et al.*, 2020).

C. gigantea has also been investigated for use in the production of paper pulp and as a source of methane in anaerobic fermentation (Schmelzer and Gurib-Fakim 2008). Leaves can be used as mulching material, and the charcoal obtained from the wood was used in gun powder and fireworks. The gynostegium of the *C. gigantea* flower is used to make sweetmeats in China and Indonesia. In Vietnam, this plant is also used as a hedge plant. (Gaur *et al*., 2013).

CONCLUSIONS

Calotropis gigantea is an important medicinal plant in Sri Lanka that has to be conserved since it is already in the Red data book of IUCN. The plant has high potential to use as a medicinal source and produce user friendly mosquito repellent. Hence, it is important to protect the plant as a native Sri Lankan species with diverse applications.

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GROWTH AND YIELD OF *Aloe vera* IN RESPONSE TO DIFFERENT ORGANIC FERTILIZERS OR MANURES

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ABSTRACT

Aloe vera is the plant belongs to family Liliaceae. Aloe vera has acquired great commercial importance globally. In Aloe vera, researches clearly show that organic manures are more efficient compared to chemical fertilizers. This study investigated the growth and yield of Aloe vera in response to different organic fertilizers or manures. This research was laid out in Randomized Complete Block Design (RCBD) in the Crop Farm, Eastern University of Sri Lanka, as a polybag experiment with five treatments and four replicates from November 2019 to March 2020. The treatments are T1 (Control), T2 (Compost), T3 (Cow dung), T4 (Commercially available organic liquid fertilizer) and T5 (Jeewamirta). In almost all the growth and yield parameters showed a significant increase by compost (T2) and cow dung (T3) treatments compared to other treatments. In those significant parameters, between compost (T2) and cow dung (T3) treatments were shown non-significance in total leaf skin weight, total root fresh weight and leaf skin weight of largest leaf. Therefore, considering the growth parameters, yield parameters, and especially economic important parameter (gel dry weight) compost and cow dung could be recommended to enhance the growth and yield in the cultivation of Aloe barbadensis.

Keywords: Aloe barbadensis, Compost, Cow dung, Fertilizers, Jeewamirta

INTRODUCTION

Word "Alloeh" gives the meaning of "shining bitter substance" in Arabic, while the Latin word "vera" gives the meaning of "true"(Christaki and Florou-Paneri, 2010). Among its 400 species of *Aloe vera* (Moghaddasi and Verma, 2011), *Aloe arborescens* and *Aloe barbadensis* are the two popular species used in commercial cultivation (Manvitha and Bidya, 2014). It mainly grows in the dry regions of America, Europe, Asia and Africa (Surjushe et al., 2008).

The modified stem of *Aloe* consists of outer dark green parenchyma entrapping thick mucilaginous colourless gel inside it. Sabats' study indicated "around 98.5-99 % moisture content, and the dry matter includes 55 % of polysaccharides, 17 % of sugars, 16 % of minerals, 7 % of proteins, 4 % of lipids and 1 % of phenolic compounds" in *Aloe vera* gel and also have many important vitamins and antioxidants in it (Sabat et al., 2018). Uses of *Aloe vera* are known for their medicinal, skincare properties, health, beauty (Surjushe et al., 2008) and food preservative properties (Manvitha and Bidya, 2014). *Aloe vera* leaf contains several medicinal activities, with hepatoprotective, immunomodulatory, anticancer, antiulcer, antioxidant, antidiabetic, antimicrobial and many other activities (Manvitha and Bidya, 2014).

Organic farming can be defined as an "ecological production system that promotes and enhances biological cycle and biodiversity in soil, crop and livestock. It is based on minimal use of off-farm inputs and management practices that restore, maintain and enhance ecological harmony" (Winter and Davis, 2006). Various researches have proved that composted organic matters can be used as growing media (Lucia, 2014). Therefore *Aloe vera* is tested with different organic fertilizers or manures in this study.

Indian farmers use Jeewamirta as a liquid organic fertilizer. It release nutrients more slowly and is kept in the soil for a longer period, accordingly, it makes sure longer residual effects, increased crop yield, and enhanced root development. It contains beneficial microorganisms, macronutrients, essential micronutrients, essential amino acids, growth-promoting factors like GA, IAA and many vitamins (Fazeel et al., 2019). Jeewamirta activates the soil microorganisms and maintains soil productivity (Jayappa and Narayana, 2013).

Bio-fertilizer is another organic fertilizer, which means the inoculation of microorganisms capable of converting the non-usable form nutrient elements to usable form through the biological process (Bandara et al., 2019). Bio-fertilizers are eco-friendly and support developing organic agriculture. To increase crop productivity Bio-fertilizers can deliver an eco-friendly weapon to farmers (Moorthy et al., 2012). Activities of beneficial soil organisms are enhanced by the addition of Bio-fertilizer though it improves soil structure. Provision of the nutrient through the Bio-fertilizer sustains the fertility and moisture of soil (Bandara et al., 2019).

Considering the uses and values of *Aloe vera*, it is necessary to find out a suitable recommendation for *Aloe vera* fertilization. Therefore, the present study was carried out to determine the growth and yield of *Aloe vera* in response to different organic fertilizers or manures.

METHODOLOGY

The research was carried out from November 2019 to April 2020 in the crop farm. It is situated in Eastern University, Sri Lanka, with a latitude of 7° 43' N and a longitude of 81°42' E. This place falls under the agro ecological zone of low country dry zone (DL2b) at an altitude of 10 m above the mean sea level. Type of soil of this area is sandy regosol, mean annual rainfall is 1400 mm to 1680 mm and temperature is 30° to 36°C.

In this experimentation, black colour polybags of 30 cm diameter and 30 cm height were used. To drain excess water, four holes were made at the base of each of these polybags. Bags were filled with top soil leaving 5 cm from the top of the polybag.

Three months old *Aloe vera* seedlings were used for the experiment. Uniform seedlings were obtained from a home garden in Kiraankulam, Batticaloa, Sri Lanka.

Each pot was planted with one seedling. Plants were irrigated once a day in the evening. Fertilizers were applied according to the treatments. Jeewamirta was sprayed (570 l/ha) in liquid form at a two-week interval from the first week after transplanting until the destruction of the plants. Compost and cow dung were incorporated with soil in solid form at the rate of 10 tons/ha as basal application. Commercially available organic liquid fertilizer (Eco Green Plus) was sprayed (1 l/acre) three days, one month, two months and three months after transplanting as per the recommendation. Hand weeding was done in two-week intervals. The polybags were maintained weeds-free until the final harvest. For the control of pests and diseases, neem extract was applied once in two weeks.

Jeewamirta

Preparation of Jeewamirta liquid fertilizer

10 kg fresh cow dung, 10 l cow urine, 2 kg jaggery, 2 kg pulse powder were added to plastic containers. Then 200 l of clean water and hand full of fertile soil were added to that plastic container and mixed well with all ingredients. After that container was covered by cotton cloth for three days for fermentation. It was stirred every morning and evening to activate microbes. After three days, it was ready to apply after filtered through a cotton cloth and it can be used for up to 8 days. Before the application, the organic mixture was diluted ten times with water.

Treatments

Five treatments and four replications were arranged in Randomized Complete Block Design (RCBD) for this experiment.

Table 1: Treatments' code and description

Treatment code	Description
T1	Control (without fertilizers)
T2	Application of Compost (10 t/ha)
T3	Application of Cow dung (10 t/ha)
T4	Application of commercial organic liquid fertilizer (1 l/acre)
T5	Application of Jeewamirta (570 l/ha)

Before two days of transplanting, compost was added to polybags at the level indicated in the table above and completely incorporated with the soil. The compost was made up of vegetable waste, sawdust, locally available organic wastes, garden prune, and cow dung. Before two days of transplanting, cow dung was added to polybags and completely incorporated with the soil at the level indicated in the table above. Commercial organic fertilizer was diluted 160 times with water and applied for this experiment at a recommended level, as indicated in the table above, after three days, one month, two months, and three months of transplanting as field application. Prepared Jeewamirta was diluted ten times with water and applied for this experiment at a recommended level as indicated in the table above. From the first week after transplanting Jeewamirta was applied at a two-week interval until the destruction of the plants. Required liquid fertilizer (Eco Green Plus) was collected from the main laboratory in Naula, Dambulla, Sri Lanka and brought to the farm. Liquid fertilizer was stored in a cool and well-ventilated area for better provision of the oxidative environment to contain microorganisms in the end product, which is favourable for microorganisms.

Measurements

Collection and preparation of sample

The plants were harvested 20 weeks after transplanting. The whole plants were carefully uprooted without damaging any roots by washing them in water and the measurements were taken.

The plants were washed to get rid of the foreign materials and dirt sticking to them. The spines in the leaves and the thick dark green outer skin (epidermis) were peeled out manually from the thick colourless parenchyma (or gel fillet) and were cut into small slabs with a stainless-steel knife. Those were stored in airtight container until the parameters were taken to avoid moisture loss and contamination.

Convective drying of Aloe vera samples

Quality of the *Aloe vera* is most affected by the drying temperature; therefore, the variation of nutritional qualities concerning temperature is considered here. The *Aloe vera* samples were loaded on the drying trays at a load density of 15 kg m^{-2}. The samples were dried at 70ºC. The drying process is ceased until no weight reduction or constant weight is attained.

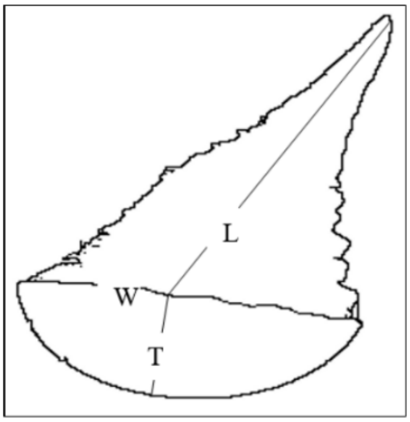

Fig. 1: Approximate geometry of Aloe vera leaf

The measurements (leaf length, leaf width, leaf thickness, total leaf weight, leaf skin weight, gel weight, leaf skin dry weight, gel dry weight, root weight, root dry weight and number of lateral roots) were taken after destructing the plant after the final harvest. Leaf length was measured using a meter ruler. Leaf width and leaf thickness were measured by a Vernier caliper (Yamayo, Model: 101-300, Japan). Leaf volume was calculated by the formula given by Sabat et al. (2018) as below:

$$\left[V = \frac{L}{12}\,\pi\,\mathrm{W\,T}\right]$$

Where;
V= Leaf volume
L= Leaf length
W= Leaf width
T= Leaf thickness

The leaf with the highest volume is considered as the largest leaf of the plant. The number of lateral roots were counted. Leaf skin and the roots were cut into small pieces and they were dried at 105°C until a constant weight was gained by using the oven (Gemmy, Model: YCO-010, Taiwan). Leaf skin fresh and dry weight, gel dry weight, and weight of roots were measured using an electronic balance (Hanon, Model: FA2204, China). The moisture content was determined by using the method described in AOAC (1990). It was calculated by the formula given by Sabat et al. (2018) as below:

$$\left[Moisture\ content(\%) = \frac{Weight\ of\ water\ present\ (g)}{Weight\ of\ dry\ matter\ present\ (g)} \times 100\right]$$

Analysis of data

Data were statistically analysed by SAS 9.04 and the mean comparison within treatments was achieved by the Duncan Multiple Range Test at a 5 % significant level.

RESULTS AND DISCUSSION

Gel Parameter

Total gel dry weight

The data showed that the compost application had the highest total gel dry weight compared to control plants. The maximum total gel dry weight was in T2, after that T3, T4, T5, and minimum total gel dry weight was in T1 at 20 WAP.
This considerable rise of total gel dry weight because of the compost treatment to the T2. It may be due to compost increase soil fertility, cell division and cell elongation. This enhanced results due to the faster decomposition of organic manure. Compost increases protein synthesis by increasing the nitrogen availability and ultimately resulting more dry matter production, which was in agreement with Babalad (2005). The high amount of C in the compost provides agronomic and environmental benefits by improving the soil physical properties (Evanylo et al., 2008). Controlled but regulated supply of nutrients from compost increased N, P, and K uptake. It helps higher growth and yield in *Aloe vera*. A similar finding has been reported by Lucia (2014).

Total gel moisture content (DB)

There is no significant difference between treatments; by considering the mean value, maximum total gel moisture content was in T5 after that T3, T1, T2, and minimum total gel moisture content was in T4 at 20 WAP.
This rise of total gel moisture content is because of the treatment of Jeewamirta to the T5. Jeewamirta holds the water well and similar finding has been reported by Devakumar et al. (2014).

Leaf Parameters

Total leaf skin dry weight

The data showed that the compost application had the highest total leaf skin dry weight compared to control plants. The maximum total leaf skin dry weight was in T2, after that T3, T4, T5, and minimum total leaf skin dry weight was in T1 at 20 WAP.

This considerable rise of total leaf skin dry weight because of the compost treatment to the T2. It may be due to compost increase soil fertility, cell division and cell elongation. It may be due to compost increase soil fertility, cell division and cell elongation. This enhanced results due to the faster decomposition of organic manure. Compost increases protein synthesis by increasing the nitrogen availability and ultimately resulting more dry matter production, which was in agreement with Babalad (2005). Compost application build-up N in soil and it contribute to the surge of nitrates in those treatments over time (Hepperly et al., 2009). Similar results were observed by Lucia (2014) in Aloe vera.

Leaf thickness of the largest leaf

This data showed that the compost application had the highest leaf thickness of the largest leaf compared to control plants. The maximum leaf thickness of the largest leaf was in T2, after that T3, T5, T4, and minimum leaf thickness of the largest leaf was in T1 at 20 WAP.

This considerable rise of leaf thickness of the largest leaf is due to compost application to the T2. Compost provided better results due to increased cell division and elongation without hindering the nutrient uptake (Hasanuzzaman et al., 2008). A similar finding has been reported by Hasanuzzaman et al. (2008) and Patke et al. (2018).

Leaf width of the largest leaf

The data showed that the compost application had the highest leaf width of the largest leaf compared to control plants. The maximum leaf width of the largest leaf was in T2, after that T3, T4, T5, and minimum leaf width of the largest leaf was in T1 at 20 WAP.

This considerable rise in leaf width of the largest leaf is because of the compost treatment to the T2. Compost increase soil fertility, cell division, cell elongation and plant growth. A similar trend was observed by Hasanuzzaman et al. (2008) in Aloe vera. Hasanuzzaman et al. (2008) observed maximum leaf width (5.11 cm) by using cow dung. The minimum average leaf width was recorded in the control. This result was supported by Bates (1971) and Saha et al. (2005).

Total leaf skin weight

The data showed that compost and cow dung had the highest total leaf skin weight compared to control plants. The maximum total leaf skin weight was in T2, after that T3, T5, T4, and minimum total leaf skin weight was in T1 at 20 WAP.

This considerable rise in total leaf skin weight because of the compost treatment to the T2 and cow dung to T3. It may be due to compost increase soil fertility, cell division and cell elongation. This result was supported by the findings of Hasanuzzaman et al. (2008), Pichgram (1987) and Saha et al. (2005). Furthermore, a similar finding has been reported by Patke et al. (2018).

Leaf skin weight of the largest leaf

The data showed that compost and cow dung had the highest leaf skin weight of the largest leaf than control plants. The maximum leaf skin weight of the largest leaf was in T2, after that T3, T5, T1, and minimum leaf skin weight of the largest leaf was in T4 at 20 WAP.

This considerable rise of leaf skin weight of the largest leaf is due to the compost application to the T2 and cow dung to T3. It may be due to compost and cow dung increasing soil fertility, cell division and cell elongation. This result was supported by the findings of Hasanuzzaman et al. (2008), Pichgram (1987) and Saha et al. (2005). And similar finding has been reported by Lucia (2014) and Patke et al. (2018)

Leaf volume of the largest leaf

There is no significant difference between treatments; by considering the mean value maximum leaf volume of the largest leaf was in T2, after that T3, T5, T4, and the minimum leaf volume of the largest leaf was in T1 at 20 WAP.

This rise of leaf volume of the largest leaf is due to the compost treatment to the T2. Compost provided better results due to increased cell division and elongation without hindering the nutrient uptake (Hasanuzzaman et al., 2008).

Root Parameters

Total root fresh weight

The data showed that compost and cow dung had the highest total root fresh weight compared to control plants. The maximum total root fresh

Table 2: Growth and yield characters of *Aloe vera* in response to different organic fertilizers or manures.

Treatment	Total gel dry weight (g)	Total leaf skin dry weight (g)	Leaf thickness of the largest leaf (cm)	Leaf width of the largest leaf (cm)	Total leaf skin weight (g)	Total root fresh weight (g)
Control (T1)	0.1416 ± 0.0461^{c}	0.4623 ± 0.0273^{c}	0.9375 ± 0.1143^{b}	2.5375 ± 0.3091^{c}	33.09 ± 7.13^{b}	10.01 ± 0.62^{b}
Compost (T2)	0.5937 ± 0.1031^{a}	2.4863 ± 0.3647^{a}	1.4250 ± 0.0968^{a}	4.1625 ± 0.3896^{a}	80.66 ± 14.57^{a}	17.01 ± 2.09^{a}
Cow dung (T3)	0.4251 ± 0.0970^{b}	1.6086 ± 0.3912^{b}	1.2000 ± 0.0890^{ab}	3.6750 ± 0.3256^{ab}	78.38 ± 4.24^{a}	14.86 ± 1.05^{a}
Commercial organic fertilizer (T4)	0.2746 ± 0.0433^{bc}	0.7271 ± 0.1666^{c}	1.0125 ± 0.1008^{b}	3.2250 ± 0.3660^{bc}	38.12 ± 6.05^{b}	9.68 ± 0.89^{b}
Jeewamirta (T5)	0.2418 ± 0.0523^{c}	0.6654 ± 0.1084^{c}	1.0375 ± 0.0375^{b}	3.2125 ± 0.1297^{bc}	50.33 ± 5.16^{b}	15.12 ± 1.54^{a}
F-test	*	*	*	*	*	*

The value represents the mean ± standard error of four replicates.
'' and 'ns' represents significant at and non-significant at $P<0.05$, respectively.*
Means followed by the same superscripts in the same column are not significantly different at 0.05 probability level according to DMRT.

Table 3: Growth and yield characters of *Aloe vera* in response to different organic fertilizers or manures

Treatment	Leaf skin weight of the largest leaf (g)	Total gel moisture content (DB) (%)	Leaf volume of the largest leaf (cm^3)	Number of total lateral roots	Total root dry weight (g)
Control (T1)	8.23 ± 1.82^{b}	44173.65 ± 6192.53	18.06 ± 6.07	14.25 ± 1.93	0.4083 ± 0.0283
Compost (T2)	20.33 ± 4.20^{a}	36208.96 ± 10336.09	58.34 ± 12.75	20.25 ± 1.65	0.9650 ± 0.3241
Cow dung (T3)	19.39 ± 1.28^{a}	44358.73 ± 8181.69	38.50 ± 2.36	16.00 ± 3.58	0.7546 ± 0.1434
Commercial organic fertilizer (T4)	7.94 ± 1.08^{b}	31842.07 ± 4229.70	24.97 ± 4.52	15.25 ± 2.17	0.4526 ± 0.1004
Jeewamirta (T5)	11.60 ± 1.52^{b}	51730.35 ± 11870.43	27.01 ± 2.30	11.75 ± 1.80	0.6558 ± 0.0982
F-test	*	ns	ns	ns	ns

The value represents the mean ± standard error of four replicates.
'' and 'ns' represents significant at and non-significant at $P<0.05$, respectively.*
Means followed by the same superscripts in the same column are not significantly different at 0.05 probability level according to DMRT.

weight was in T2, after that T5, T3, T1, and minimum total root fresh weight in T4 at 20 WAP.
This considerable rise of total root fresh weight because of the compost treatment to the T2, cow dung to T3 and Jeewamirta to T5. It may be due to Jeewamirta having the highest bacterial and actinomycetes population, which promotes root growth and root development (Fazeel et al., 2019). Compost increases total porosity and changes the distribution of pore sizes in soils (Celik et al., 2017). Maximum root fresh weight (9.8 g) was observed by Saha et al. (2005) in *Aloe vera*.

Total root dry weight

There is no significant difference between treatments; by considering the mean value, the maximum total root dry weight was in T2 after that T3, T5, T4, and minimum total root dry weight was in T1 at 20 WAP. This rise of total root dry weight because of the compost treatment to the T2. Compost increases total porosity and changes the distribution of pore sizes in soils (Celik et al., 2017). Furthermore, it may be due to compost positively responsive with a higher amount of compost.

Number of total lateral roots

There is no significant difference between treatments; by considering the mean value maximum number of total lateral roots was in T2 after that T3, T4, T1, and the minimum number of total lateral roots was in T5 at 20 WAP.
This increase in the number of total lateral roots is due to the compost application to the T2. It may be due to compost providing the essential nutrient which resulted the maximum cell growth. Compost increases total porosity and changes the distribution of pore sizes in soils (Celik et al., 2017).

CONCLUSIONS

Aloe vera has a strong demand in both national and international markets due to its uses and values and it is more responsive to nutrients. Organic fertilizer plays an important role in increasing production, improving quality and sustaining soil fertility and organic matters can be used as growing media. Therefore *Aloe vera* is tested with different organic fertilizers or manures in this study. In almost all the growth and yield parameters showed a significant increase by compost (T2) and cow dung (T3) treatments compared to other treatments. In those significant parameters, between compost (T2) and cow dung (T3) treatments were shown non-significance in total leaf skin weight, total root fresh weight and leaf skin weight of largest leaf. Therefore, considering the growth parameters, yield parameters, and especially economic important parameter (gel dry weight) compost and cow dung could be recommended to enhance the growth and yield in the cultivation of *Aloe barbadensis*.

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NUTRITIONAL, MICROBIAL AND SENSORY EVALUATION OF JAM STORED UNDER AMBIENT CONDITION USING PECTIN ISOLATED FROM POMELO (*Citrus maxima)* PEELS

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ABSTRACT

Fruit peels are agro wastes that may be considered as raw materials, however despite of their pollution and hazardous aspects, wastes from fruit processing industries have a great potential for regeneration into products of significant value as raw materials for other industries, while considering the fruit processing industries citrus is one of the major processed fruits, there is a substantial prospective to utilize citrus peels to isolate value-added ingredients as pectin. This study focused to use pomelo peels, as a source of pectin and its utilization in the fruit jam production with different blends of pectin and sugar levels and assess its nutritional, sensory qualities and stability during ambient storage condition. Quality parameters of jam formulations were assessed using standard AOAC methods. Organoleptic attributes were assessed by 30 semi-trained panelists using a seven-point hedonic scale. The foremost preferred jam formulations were stored and analyzed in ambient temperature at 70-75% RH for 12 weeks period. Quality parameters during storage of watermelon jams revealed the increasing trend in total soluble solids, titratable acidity and moisture content. Moreover, the declining trend in pH, and vitamin C content. Based on the quality parameters, sensory analysis and microbiological studies, the watermelon jam with 4.2 g (14% dry basis) extracted pomelo pectin and 65 g (65ºBrix) sugar, could be stored in ambient temperature for 12 weeks period of time with none significant change in quality characteristics.

Keywords: Agro waste, Citrus, Organoleptic, Pectin, Peels, Quality parameters

INTRODUCTION

Fruits are widely used as a magnificent source of phytochemicals and micro nutrients. Most of the fruits are seasonal and perishable, this needs some processing to preserve the nature of the fruits to maintain and preserve quality attributes and provide various products such as candies, jellies, jam and juices throughout the year, particularly in the off-seasons. Jams were produced as an attempt to conserve the fruit consumption (Baker *et al*., 2005). Jam is an intermediate moisture fruit-based product developed from fresh fruits by cooking fruit pulp with sugar, gelling agent, citric acid and other additives. According to the fruit products order specification, jam is made by cooking the pulp with the required amount of sucrose to a thick consistency that holds the fruit tissues in place (55% of sugar should be used for each 45% of fruit pulp) and the finished jams must have a brix value of at least 68.5°Brix (SLS 265:2011). Jam has substantial nutritional value with the specified level of vitamin C content, phenolic compounds, antioxidant and other nutrients. The crucial constituents within the preparation of jam are pectin, sucrose and acids in the appropriate proportion for accepted gel formation.

Gelling agents are food additives that provides the food with texture by the development of a gel substance. Typical gelling agents include alginic acid, sodium alginate, locust bean gum, starches, pectin, gelatin and agar. Since, 200 years pectin has been used in the jelly manufacturing industry (Willats *et al*., 2006), but only citrus albedo and apple pomace, sources have been commercially acceptable, as raw materials (Iglesias and Lozano, 2004).

Citrus fruits, peel as an adequate source of pectin, peel consists of two parts the epicarp and mesocarp. The part that is called epicarp is the outermost coloured surface of the peel, while the part that is called mesocarp is the soft white middle layer of the peel (Davies and Albrigo, 1994). Pectin is being isolated through several methods. Usually, commercial pectin is extracted during a several physico-chemical processes characterized by an extraction and precipitation step respectively, using hot dilute mineral acid and recovery through an overnight alcohol precipitation (Mollea *et al*., 2008). Pectin is commercially available as white to brown powder, made from citrus peels, that is used as gelling agent in foods such as jams, jellies, marmalades and ketchups (Kale and Adsule, 1995).

Watermelon (*Citrullus lanatus*) is an important cucurbitaceous crop, contains 92% water, with small amounts of protein, fat, vitamins and minerals. The vital nutritional components of the fruits are carbohydrates, especially sucrose, fibre, vitamin A and lycopene content and excellent source the Vitamin C. Sugar boosts the network formation and enhances gelling in jams, jellies and preserves through attraction of water and yanking it away from the pectin. Sugar is a dehydrating agent helps to prevent the growth of mainly molds and bacteria. Sugar provides jam its long-term keeping qualities. Citric acid helps the pectin to set. The optimum low pH is between 2.8 and 3.3 for the formation of gel (Apsara and Puspalatha, 2002). Jams and jellies are sugar containing food preserves that are possible to acquire microbial spoilage immediately after preparation. The molds and bacteria are notable spoilage sources of fruit products and their presence in the finished products beyond the permissible level are considered unfit for consumption (Vidhya and Narain, 2011).

The existence of molds in food may reduce food quality and also contamination of food with mycotoxins, which may cause allergic reactions and respiratory problems (Calado *et al*., 2014). Consumer acceptability testing in the form of well-planned sensory analysis, is an important aspect of any finished product's shelf-life evaluation, as a result the present study objectives were to assess the quality parameters, microbial safety and consumer acceptability of jam stored at ambient condition.

METHODOLOGY

Isolation of Pectin from Pomelo Peels

The pomelo peels were collected from juice processing centers in Batticalo, and the mesocarp the soft white middle layer of the peels was carefully removed and sun dried for 4 days, till their moisture content was negligible. The dried mesocarps were ground and fine particles were sieved with a mesh size of 60 and stored in polyester metalized bags in air tight glass containers at room temperature for further use. The peel pectin extraction procedure was followed by the method given by Methacanon *et al*. (2014). Comparing to other acids in extraction procedure the best solvent for extracting pectin is Hydrochloric acid (Kalapathy & Proctor, 2001). Dried pomelo peel powder of 30 g was transferred into a 1000ml beaker containing 300 ml of 0.01N HCl. The mixture was then boiled in an 80°C water bath for 1 hr, to recover the extract, the hot acid extract was filtered through a filter funnel equipped with a two-layer of muslin cloth and squeezed, a ratio of 1:2 (1 part extractant and 2 parts ethanol) 95% ethanol was added and allowed to precipitate overnight at 4°C, Whatman No. 1 was used to filter the precipitated gelatinous pectin which was then washed with ethanol twice to remove impurities and then it was weighed after 3 hrs of drying in an air dry oven at 40°C. The dried pectin was crushed into a fine powder and stored at ambient temperature in air tight containers for further use.

Studies on Development of Watermelon Jam Using Pectin from Pomelo peels

A number of preliminary experiments were conducted to find out the appropriate amount of watermelon pulp, sugar, pectin, citric acid and to develop the recipe for the jam formulation at different combinations of sugar and pectin levels.

Development of Jam Formulations

The weight of watermelon pulp, citric acid and sugar was weighed by using an electric balance (METTER PJ-300). Initially 500 g of watermelon pulp, sugar (g/100 g) and pectin (g/100 g) were added in the ratio of 80:0, 75:1.8, 70:2.3, 65:2.8 and 60:3.3 respectively into the stainless-steel pan then stirred thoroughly and kept for 15 minutes with occasional heating until temperature reaches 105°C. Meanwhile, 2 g of citric acid was added and cooked by stirring continuously, until it reaches end point as, total soluble solid 68ºBrix (SLS 265:2011). Sheet or flake test was done to determine the end point, then strawberry essence was added at the rate of 5ml (5 ml/100 g) and mixed thoroughly. At last, the jam was removed from the fire and cooled for 5 minutes. To maintain the inner pressure (10-12 kPa) the plastic cups were sterilized by spraying water at 68-70°C to dry. Then freshly prepared jam was filled into plastic cups and sealed with lids.

Experimental Plan

The experiment consists of five treatments namely, T1- 80 g sugar and no pectin added; T2- 75 g sugar and 1.8 g pectin isolated from peels; T3- 70 g sugar and 2.3 g pectin isolated from peels; T4- 65 g sugar and 2.8 g pectin isolated from peels; T5- 60 g sugar and 3.3 g pectin isolated from peels.

The formulations such as T1, T3, T4, T5 with high sensory scores in organoleptic evaluation were chosen for storage studies and stored at room temperature for a period of 12 weeks. The nutritional quality parameters such as moisture, titratable acidity, pH, ascorbic acid and total soluble solids were assessed at 2 week intervals. After 12 weeks of storage, the findings of sensory qualities were determined.

Quality Parameters

Pectin quality characteristics such as colour, solubility in both cold and hot water and in alkali, pectin yield, ash content, moisture content and degree of esterification were analyzed as described by Ranganna (2005). Nutritional qualities of the freshly prepared watermelon jam were analyzed using recommended standard methods (AOAC, 2002). The Digital pH meter was used to determine the pH (HI HANNA Model 97150). The titratable acidity of the jam samples was measured by titrating them with standard alkaline and expressing the results as a percentage of citric acid. Hand refractrometer (Model ATOGA-S-29E) was used to measure the Total Soluble Solids (TSS). Titrimetrically, the ascorbic acid level was determined using the 2, 6 dichlorophenol indophenol dye method and moisture content was determined by the gravimetric method. During analysis each parameter were triplicated.

Microbial Analysis examination was conducted using Potato Dextro Agar (PDA) method and the total plate count was observed as the procedure mentioned below.

The peeled potato was cut into small pieces and added in 250 ml of distilled water and boiled. Weighed agar was boiled with 250 ml of distilled water until agar dissolve and placed in a 1000 ml of a flask. Then the needed amount of potato extraction and sucrose were added into the flask and stirred thoroughly. Cotton wool was used to plug the media, wrapped by using aluminum foil and media was sterilized by using an autoclave at 121ºC, 15psi for 20 minutes then allowed to cool. Petri dishes, forceps and needles were sterilized using a hot air oven at 180ºC for one hour and later allowed to cool. All the glasswares were sterilized with 70% of alcohol, then the media was poured into petri dishes, until media gets solidify kept in laminar flow. Different jam samples from T1 to T5 were placed in separate agar plates. Then petri dishes were covered and labeled accordingly. The dilutions were produced up to 10^{-4}, with the aid of laminar airflow. Incubation was done for 48 hours at 37°C, with results expressed in CFU/g. The total plate count of formulations was evaluated during a period of 1,2 and 3 months.

Sensory Evaluation

Sensory Evaluation was assessed using 30 semi-trained panelists. The sensory attributes which include colour, taste, texture, aroma and overall acceptability were assessed. The test was conducted in two session's morning 9.00 - 11.00 am and evening session 2.00 – 4.00 pm. A structured questionnarie was used for the sensory evaluation. To examine the degree of liking (7) and disliking (1) for the preference of the jam formulation, seven-point of hedonic scale was used following the storage period.

Statistical analysis

The trail was conducted in a Complete Randomized Design with triplicates of each formulation. The physicochemical data's parameters were subjected to analysis of variance (ANOVA) at a significance level of 5%. Duncan's Multiple Range Test (DMRT) was used to separate the means for storage studies. The Kruskal-Walli's test was used to assess data relating to sensory evaluation. Statistical Analysis System software statistical package was used for both nutritional and organoleptic analysis.

RESULTS AND DISCUSSION

Quantitative and Qualitative analysis of Pectin

The extracted pomelo pectin characterized in terms of pectin colour, solubility in water, and alkali (NaOH), pectin yield, ash content, moisture content, and degree of esterification, as these properties determines the applicability of pectin for a variety of applications.

Pectin content extracted from pomelo peel using HCl was 14% (dry basis) that is comparable acceptable with other commercial sources such as sugar beet and apple pomace (Ismail *et al*., 2012). Higher yield was obtained by using HCl at 80ºC, 60 min, this value remained in close accord with the findings provided by (Devi *et al*., 2014), extracted pectin from pomelo peels by two distinct acids (HCl and nitric) and at three different temperatures (60, 70 and 80ºC), time (30, 45 and 60 min).

Moisture content of pomelo pectin was below 11%. These values were lower than the pectin extracted from lemon pomace (Ismail *et al*., 2012). Stability and functionality of pectin is determined by the moisture content, the rate of hydration of pectin in gelation is affected by the moisture content.

The ash content indicates the inorganic impurities in the pectin. It was found to be 6.6% of ash content of pectin extracted using HCl which is 16.4% for commercial pectin. Higher ash content interferes in gelation (Devi *et al*., 2014). Therefore, it is important to have lower ash content is more favorable for gel formation. Degree of esterification of pomelo pectin was 67.64%, as a result it's classified as high methoxy pectin.

Nutritional Analysis of watermelon pulp primarily to develop the standard recipes of watermelon jam. Table 1 shows the Nutritional Analysis of watermelon pulp (*Citrullus lanatus*).

Table 1: Chemical Composition of Watermelon Pulp

Chemical composition	Watermelon pulp
Titratable Acidity (as % citric acid)	0.22±0.12
Total soluble Solids (°Brix)	14.56±0.01
pH	6.52±0.01
Vitamin C content (mg/100ml)	6.81±0.01
Total sugar (%)	6.25±0.01

The values are means of triplicates ± standard error

Quality parameters of Jam Formulations during Storage

Titratable Acidity

Titratable acidity is a crucial quality indicator refers to any acid that can lose protons in an acid-base reaction. If the acid level is too low, the product may be bland and unappealing (Rahman and Moshiur, 2017). Acidity is the direct proportional indicator of a product's ability to maintain its quality against microorganism growth. Figure 1 shows the changes in titratable acidity of the watermelon jam during storage.

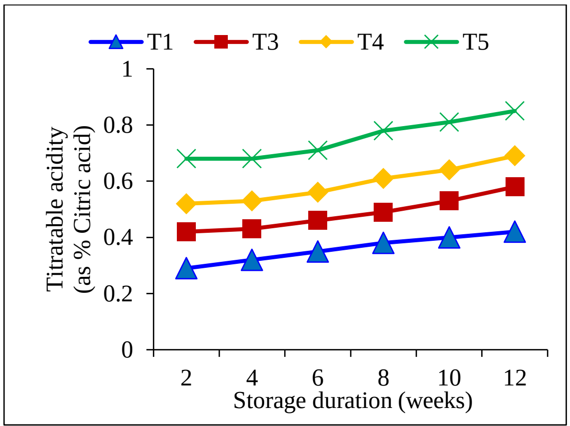

Figure 1: Changes in Titratable Acidity in Watermelon Jam during Storage

The values are means of triplicates ± standard error

(T1- 80g sugar and no pectin added; T3- 70g sugar and 2.3g pectin extracted from lemon peels; T4- 65g sugar and 2.8g pectin extracted from lemon peels; T5- 60g pectin and 3.3g pectin extracted from lemon peels).

The overall result showed that, the acidity of watermelon jam significantly ($p<0.05$) showed an increment throughout the storage period at a highest value of 0.89% was observed in jam with 3.3g pectin after 12 weeks. The lowest value of 0.27% was showed in jam with no pectin added after two weeks. This might result from formation of carboxylic acids from alcohol which was produced from sugar and this might be due to the addition of acid into the watermelon jam.

pH

The result indicates that the pH of watermelon jam decreased with increasing duration of storage in all the formulations. There was a significant ($p<0.05$) reduction in pH during the storage period at ambient temperature (30°C). It was observed that the maximum pH (3.94) in the jam with no pectin added formulation in the 2ndweek and the least pH (3.31) was observed in T5 (3.3g pectin) at the end of storage. The pH of preserved products plays a role as a preservative (Ahmed *et al*., 2016). The result pertaining to the response of storage duration and different treatments of watermelon jam on pH are present in Figure 2.

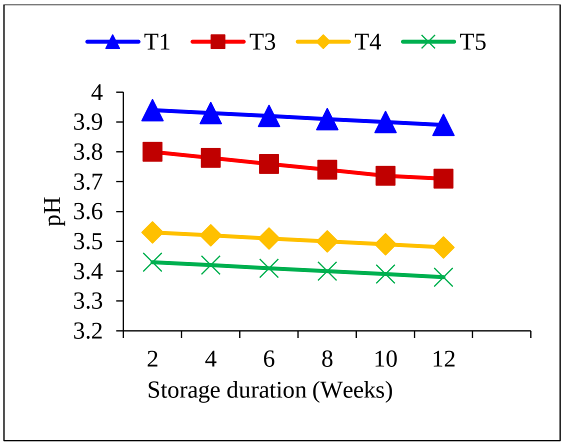

Figure 2: Changes in pH of Watermelon Jam during Storage

The values are means of replicates ± standard error

(T1- 80g sugar and no pectin added; T3- 70g sugar and 2.3g pectin extracted from lemon peels; T4- 65g sugar and 2.8g pectin extracted from lemon peels; T5- 60g pectin and 3.3g pectin extracted from lemon peels).

It's possible the reduction in pH might be due to the formation of acids from added sugar during the storage. The findings are consistent with those of others who evaluated the pH which indicates declining tendencies over time intervals. Similar reduction in pH has been reported by Hussain *et al*. (2010), in watermelon lemon jam during storage

Ascorbic acid Content

The ascorbic acid content of the watermelon jam showed a reduction significantly ($p<0.05$) with the storage period (Figure: 3). According to DMRT, the vitamin C content had a significant ($p<0.05$) reducing trend to the storage in all formulations. Ascorbic acid is sensitive to oxygen, heat and light, it is easily oxidized, in the presence of oxygen by both enzymatic and non-enzymatic catalyst from ascorbic acid to dehydroascorbic acid.

The findings are consistent with those of Eskin (1979) and Land (1962) who found that decrease in ascorbic acid content of sample could be attributed to both non oxidative and oxidative changes during storage. The highest mean value 5.29mg/100ml for ascorbic acid was observed in T5 (Jam with 3.3g pectin) in the 2nd week and the least mean value 4.21mg/100ml was observed in T3 (Jam with 2.3g of pectin) in 12th weeks.

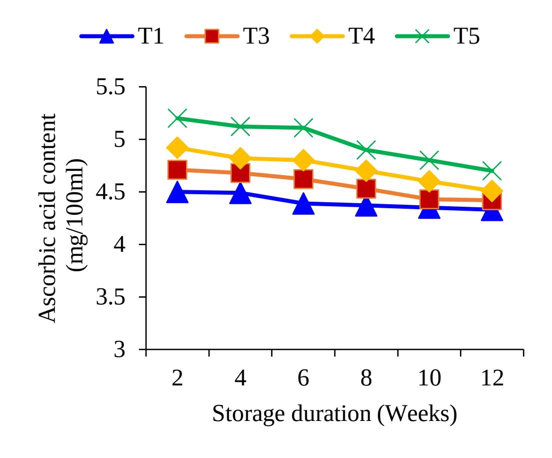

Figure 3: Changes in Ascorbic acid Content of Watermelon Jam during Storage

The values are means of replicates ± standard error

(T1- 80g sugar and no pectin added; T3- 70g sugar and 2.3g pectin extracted from lemon peels; T4- 65g sugar and 2.8g pectin extracted from lemon peels; T5- 60g pectin and 3.3g pectin extracted from lemon peels).

Moisture Content

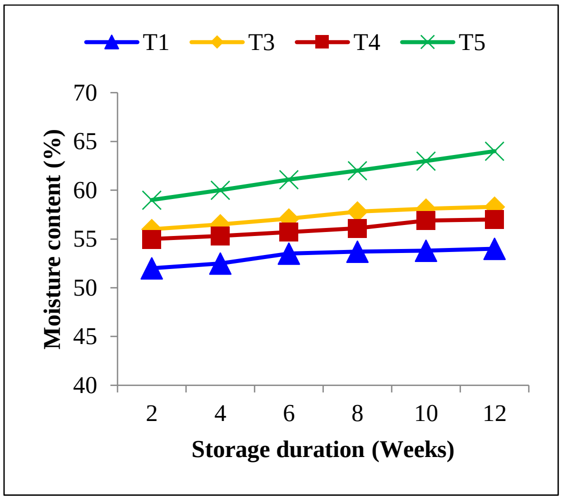

Figure 4: Changes in Moisture Content of Watermelon Jam during Storage

The values are means of replicates ± standard error

(T1- 80g sugar and no pectin added; T3- 70g sugar and 2.3g pectin extracted from lemon peels; T4- 65g sugar and 2.8g pectin extracted from lemon peels; T5- 60g pectin and 3.3g pectin extracted from lemon peels).

The moisture content of less than 10% is responsible for the state of non-deterioration in dehydrated food (Ranganna, 2005). In all jam formulations, moisture content increased significantly ($p<0.05$) throughout the storage period extending a maximum value of 66.4% was observed in jam with 60g sugar at the end of 12 weeks storage period shown in Figure 4. The minimum moisture content observed in T5 (Jam with 80g sugar) value is 53.9%.

The moisture content gradually showed an increment during storage may be due to the hydrolysis of sugar into alcohol, carbon dioxide and water. Our findings are closely in accordance with the results attributed by Yousif and Alghamdi (1999) in date jelly.

Total Soluble Solids (TSS)

The results observed during the storage period of watermelon jam formulations are shown in Figure 5. According to DMRT, there was a significantly ($p<0.05$) increment in Total Soluble Solids among treatments. This could be due to the degradation of polysaccharides into simple sugars during storage, as well as the conversion of the insoluble fraction into a soluble fraction.

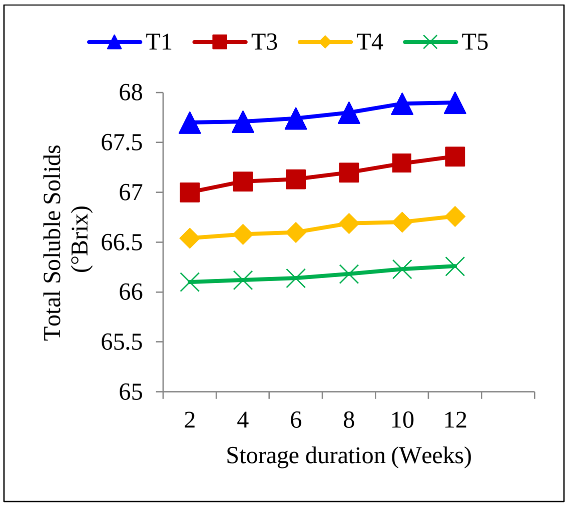

Figure 5: Changes in Total Soluble Solids (°Brix) of Watermelon Jam during Storage

The values are means of replicates ± standard error

(T1- 80g sugar and no pectin added; T3- 70g sugar and 2.3g pectin extracted from lemon peels; T4- 65g sugar and 2.8g pectin extracted from lemon peels; T5- 60g pectin and 3.3g pectin extracted from lemon peels).

These findings were in agreement reported by Khan *et al.* (2012) with the increase in TSS of pear apple jam during storage, similar findings were in agreement reported by Shakir *et al.* (2007) with the increase in TSS of watermelon and lemon jam during storage. The formulation which had 80g of sugar showed a highest value (68.17°Brix) for TSS in the 12th week of storage period. The lowest mean value of (66.10°Brix) was observed in 2nd week of the storage period which had lowest of sugar content in the formulation.

Microbiological Test for Jam formulations

The total plate count for all the samples ranges from 8.45×10^4 - 32×10^4 cfu/g reflecting the significant ($p<0.05$) differences among samples The total plate counts do not exceed the standard ($\times10^6$ cfu/g)

(ICMSF, 2002). The reduction in the microbial levels may be due to the intense heat application involved in jam production as well as addition of sugar and citric acid while processing, sugar and acid reduces the pH and makes the environment unfavorable for microorganism growth.

Sensory Analysis of Watermelon Jam Following at the End of Storage

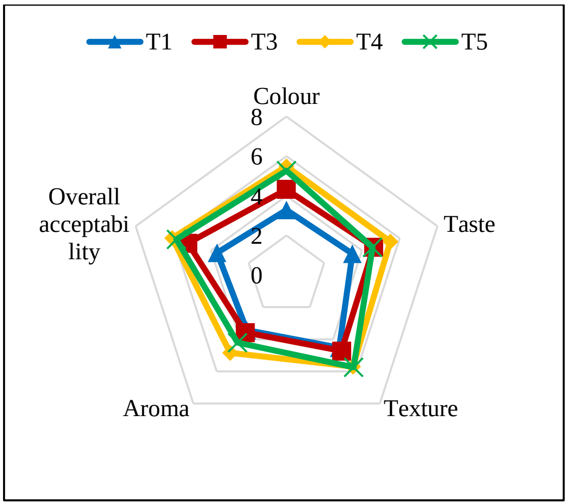

Figure: 7 Sensory Analysis of Watermelon Jam during Storage

The values are means of triplicates ± standard error

(T1- 80g sugar and no pectin added; T3- 70g sugar and 2.3g pectin extracted from lemon peels; T4- 65g sugar and 2.8g pectin extracted from lemon peels; T5- 60g pectin and 3.3g pectin extracted from lemon peels).

In this investigation, organoleptic scores reduced while increasing the storage period at room temperature 30±1°C. Sensory scores are given in Figure 7. The sensory scores with respect to colour of all jam formulations revealed a slight decrement after 12 weeks of storage (Figure 7). According to the mean value of sensory scores the highest mean value for colour was observed in formulation T4 (2.8 g pectin and 65 g sugar). These jam formulations had natural colour pigments. In storage it could be attributed to enzymatic or non-enzymatic browning (Maillard reactions). Figure 7 shows a general decrease in taste score of watermelon jam formulations. The watermelon formulations containing (2.8g pectin and 65g sugar) showed the highest mean value 4.72 for taste and formulation with (80 g sugar and no pectin added) had the least mean value score 3.49 for taste score, oxidation of ascorbic acid into dehydroascorbic acid and tannins to gallic acid, increase the acidity of the jam creates sourness.

Figure 7 shows a general decrease in texture score of watermelon jam formulations during storage. The watermelon formulations containing (3.3 g pectin and 60 g sugar) had the highest mean value score of 5.01 and formulation containing (80 g sugar and no pectin added) had the least mean value score of 4.52 for texure, the possible reason for the loss of texture score it is possible that this is due to pectic acids, which are then converted to sugars galacturonic acids. According to the results obtained for aroma shown in Figure 7, the mean scores for aroma revealed that there was significant decrement in the aroma of watermelon jam after the storage period of 12 weeks.

The reduction in aroma during storage could be due to the initial processing of jam cause losses of volatile aromatic compounds. Figure 7 shows a general decreasing trend in overall acceptability. The mean scores for overall acceptability revealed that there were significant reductions in overall acceptability of watermelon jam after a storage period of 12 weeks. A decrease in overall acceptability might be due to loss of appearance, flavor uniformity in food products during storage.

CONCLUSIONS

The current findings shown that pomelo peel is an adequate source of pectin, the natural biodegradable polymer. Pomelo pectin contains a large quantity of pectin, making it a viable commercial source for jam manufacturing. Jams with low gel strength can be improved by adding isolated pomelo peel pectin during processing to get the desired gel strength (Mollea et al., 2008). The watermelon jam prepared with 65 g (65 °Brix) sugar and 4.2 g (14% dry basis) pectin is the best formulation novel biomaterial for commercial preparation, and could be stored at ambient condition 30±1 °C and 70-75% RH for 12 weeks without any significant changes in the quality, sensory and microbial characteristics.

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NATURAL RUBBER TIRE WASTE CHARCOAL (NRTWC) ON ORGANIC CARBON MINERALIZATION IN TEA GROWING SOILS

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ABSTRACT

The aim of this study was to assess the impact of natural rubber tire waste charcoal (NRTWC) amendment on carbon mineralization in tea cultivated soils in low country wet zone of Sri Lanka. NRTWC was applied at different rates (0%, 1%, 1.6%, 2.2% and 2.8% w/w) and carbon mineralization in soil was evaluated at 10 and 20 weeks after the treatment. Representative samples were incubated for 42 days and microbial respiration was determined. During the first seven days of incubation, a rapid carbon mineralization was observed for all the treatments. A rapid decline of carbon mineralization was then observed during the period from 14th to 28th day of incubation. No changes were observed towards the end of the incubation (35 to 42 days). Significant ($P \leq 0.05$) differences among the different rates of NRTWC application were noticed during the initial stages of the incubation. However, the variations were minimal after 14th day of the incubation. The cumulative carbon mineralization ranged from 506 to 1072 mg/kg soil for the samples taken at 10 weeks after treatment. The corresponding variation changed from 506 to 1730 mg/kg soil for the samples drawn at 20 weeks after treatment. The cumulative soil carbon mineralization increased rapidly during the early stages, then decreased gradually and finally flattened out towards the end of the incubation. The application of NRTWC resulted in a substantial increase in cumulative carbon mineralization when compared with the control. As revealed by the results, NRTWC decomposes slowly and can be considered as a sound source of soil amendment which could enhance soil carbon sequestration in degraded tea soils.

Keywords: Carbon sequestration, Soil degradation, Organic matter decomposition, Soil amendments

INTRODUCTION

Most of the solid waste materials are considered to be non-degradable, thus accumulation of them is a serious environmental problem worldwide (Adhikari *et al.*, 2000). Rubber tire waste which is comprised of 12% solid wastes is notable portion of solid waste materials produced throughout the world creating environmental, economical and social problems (Ghiasi *et al.,* 2015). The global annual tire production and their disposal as waste has increased yearly due to increased in human population (Torretta *et al.*, 2015). It is estimated that globally 4 billion tons of tires are generated annually (Czajczynska *et al.*, 2017) and the number would be increased to five billion by the year 2030 (Thomas *et al.,* 2016). Rubber tire wastes are non-biodegradable due to their physical and chemical structure and remain for several years in the environment. Landfilling, retreading, gasification, incineration and pyrolysis are some of the waste tire disposal mechanisms adopted by different countries including Japan, South Korea and China (Undri *et al.*, 2013a; Duan *et al.*, 2015; Li *et al.*, 2016). Most of the developing countries stockpile the waste tires for land filling at disposal sites. It occupies substantial piece of usable lands (Alsaleh *et al.*, 2014) and provides breeding environment for pest and insects. Such tires at the disposal sites trap gases which cause floating to the top with the time (Juma *et al.,* 2006). Some countries have legally prohibited the land filling with waste tires (Junqing *et al.*, 2020). Therefore, finding feasible and cost-effective solutions to treat rubber waste tires is paramount importance.

Pyrolysis is considered as a promising approach which could minimize the tire disposal problem (Debnath *et al.*, 2018) since it lowers the negative impacts on the environment (Junqing *et al.*, 2020). It produces potentially useful products such as Pyrolysis oil comprised of aromatic and aliphatic compounds, non-condensable mixture of pyrolysis gases and solid coke (char or pyrolytic carbon black) (Williams, 2013). This pyrolytic carbon black is similar to biochar in nature (Junqing *et al.*, 2020). Due to its very slow decomposition, it ensures maintaining soil organic matter content for a longer period of time

Tea (*Camellia sinensis* L.) is an upland perennial crop grown in many tropical and subtropical regions. Soil degradation has been identified as an inescapable threat which severely restricts the productivity of most of the tea growing soils in Sri

Lanka. Soil degradation is known to be accelerated by unfavorable environmental conditions such as high rainfall and high temperature which fasten organic matter decomposition, heavy usage of agrochemicals and soil erosion. In this regards, application of organic amendments with high organic matter content is considered as feasible approach in restoration of degraded tea soils. Soil microorganisms act as an agent for soil organic matter decomposition, nutrient mineralization and main soil processes (Li *et al.*, 2018). Amending soils with high carbonaceous material may enhance the activity of soil microorganisms. Soil carbon mineralization is considered to be a direct indicator of organic matter decomposition of a soil. The present study assessed the impacts of soil amendment with different rates of Natural Rubber Tire Waste Charcoal (NRTWC) on soil carbon mineralization (microbial respiration) in tea growing soils.

METHODOLOGY

Experimental Site

Well-managed 8 years old tea plantation located at Aturaliya D.S. Division of Matara District in Southern Sri Lanka was selected for the study. The selected area comes under the agro-ecological region low country wet zone (WL2). The annual rainfall, annual mean air temperature and relative humidity of the area were 2500 mm, 22-30 ^{0}C and 80 % respectively. As listed in the United States of Department of Agriculture soil taxonomy, the soil of this area belongs to Red Yellow Podzolic (local classification) and falls under Rhodudults (Soil survey staff 2014, USDA classification). Table 1 depicts some key physico-chemical properties of the soil determined through standard methods.

Hydrometer method (Gee and Or, 2002) was used in determining soil texture and soil pH was measured using (1:2.5 soil: water) pH meter (HI 98127 HANNA). Walkley and Black method (Nelson and Sommer, 1996) was used in calculating organic carbon (OC) content and thereby determined the organic matter (OM) content using the formula (OM% = 1.721 × OC). Exchangeable K^+ was extracted by ammonium acetate (NH_4OAC) and determined using a flame photometer (Helmke and Sparks, 1996). Available soil phosphorous was extracted according to borax method (Dick and Tabatabai, 1977) and determined using a spectrophotometer (UV-VIS 160 Shimadzu). The NH_4^+ - N content was determined utilizing the Berthelot reaction (Searle, 1984) and the NO_3^- - N by sodium salicylate yellow colour method (Bremner, 1960).

Table 1: Some important physico-chemical properties of natural rubber tire waste charcoal and experimental soil. Values given here are the means (n = 4) ± standard deviation.

Properties	NRTWC	Soil
Organic matter (%)	85.8 ± 0.1	2.25 ± 0.008
Bulk density (g/cm^3)	0.2±0.005	1.39 ± 0.06
Porosity (%)	61.53±1.33	37.83 ± 1.1
pH	7.76±1.41	5.3 ± 0.4
EC (dS/m)	0.89±0.1	0.035±0.005
NO_3^- - N content (mg/Kg)	1.5±0.3	1.78 ± 0.09
NH_4^+ - N content (mg/Kg)	23.98±0.6	11.69 ± 0.14
Available P (mg/Kg)	4.96±0.05	2.15 ± 0.42
Exchangeable Ca (mg/Kg)	416.29±57.22	146.52 ± 6.79
Exchangeable K (mg/Kg)	358.16±7.04	14.21 ± 0.55
Exchangeable Na (mg/Kg)	82.52±3.44	9 ± 0.45

Production of natural rubber waste tire charcoal (NRWTC)

Waste tires were collected from dumping sites of tire shops from Akuressa and Kamburupitiya areas of Matara District, Sri Lanka. Excavated pit (one cubic meter) was tightly filled with waste tires. A thick metal sheet was used to seal up the chamber to limit the oxygen supply slowing down the burning process, reducing the emission of carbon monoxides (CO) which ensured good quality charcoal with higher carbon content. After about 3 and ½ hours of the pyrolysis process, the lid was removed, and some water was poured to avoid aerobic oxidation. This method is considered to be one of the oldest and simplest methods of charcoal making which is still widespread (Seijo and Teira-Brion, 2019). NRTWCs were then air dried, broken up to pieces with a hammer and crushed charcoals were passed through 250-µm mesh to remove larger particles. Some physico-chemical properties of NRTWC were determined following the standard procedures (Table 1).

Treatments

Generally, well-managed tea plantations in Red Yellow Podzolic soil should have 5% soil organic matter content (Sandanam and Coomaraswamy, 1982). The organic matter content of the present experimental soil and NRTWC were measured as 2.25% and 85.8% respectively. Therefore assuming that addition of NRTWC which contain 85.8% organic matter could increase the organic matter content of the experimental soil up to 5%, treatment calculations were done. Accordingly, there were five NRTWC application rates as 1% (20% less than requirement), 1.6% (requirement), 2.2% (20% higher than requirement), 2.8% (40% higher than requirement) and a control (without NRTWC application - 0%). These values are equal to a 21 t/ha, 33.6 t/ha, 46.2 t/ha, 58.8 t/ha and a control application rates.

Application of NRTWC

NRTWC was incorporated to the top 10 -15 cm layer of the manure circle of tea plants using hand-held shovels. The experimental area was 0.01 ha which contained 20 individual plots each having 1.5 m X 1.5 m size. A complete randomized block design was used with four replicates. NRWTC treated and untreated plots were separated with a 1.5 m wide buffer. Each experimental plot consisted of 10 tea bushes

Soil sampling

Soil samples were collected after 10 and 20 weeks of treatment application. Surface litter of the soil was removed and each plot of soil was randomly sampled using the five-point composite method throughout the entire experimental site from 0-15 cm depth using an auger. The composite soil samples were kept in polythene bags and transported to the laboratory. Then, soil samples were air-dried and sieved through a 2 mm mesh for the chemical characterization and incubation study.

Carbon mineralization

Soil carbon mineralization in the soils treated with different rates of NRTWC was assessed in a laboratory incubation conducted under dark condition for 42 days. Air dried and 2 mm sieved soil samples (50 g) were placed in gas-tight glass containers along with a vial containing 10 ml of 1 M NaOH to trap CO_2 and a vial of water to maintain the humidity. The treated and untreated soils in glass bottles were watered to adjust the moisture content to 60% field capacity before the incubation. There were three replicates per treatment. A control treatment (without soil samples) in triplicate was also included. Control and treated samples were arranged as a Randomized Completely Design. Soil was incubated at room temperature (27 ºC) and NaOH traps were replaced at 7, 14, 21, 28, 35 and 42 days after the treatment. Unreacted alkali in the NaOH traps was titrated with 0.5 M HCl to determine CO_2 -C released from the soil (Alef, 1995). Sufficient soil moisture content was maintained throughout the incubation period.

Statistical analysis

All the data derived from the experiment were subjected to analysis of variance (ANOVA) using SAS package (SAS, 1999). The Duncan’s Multiple Range Test (DMRT) was applied to test the significance of treatment means at $P \leq 0.05$.Values were given as means ± SD for the replicated samples.

RESULTS AND DISCUSSION

Periodic changes in carbon mineralization (as measured by CO_2 evolution) in soil amended with different rates of NRTWC are depicted in Figure 1 (a and b respectively for 10 and 20 weeks after application).

The highest C mineralization was observed in the soil treated with NRTWC at 2.8% followed by 2.2%. The soil which did not receive NRTWC exhibited significantly ($P \leq 0.05$) lower C mineralization compared to all the other treatments. During the early stage of incubation (1 to 7 days), a rapid C mineralization was observed in all treatments for both incubated soils. Thereafter, C mineralization was rapidly declined (14 to 28 days) and reached to a constant towards end of the incubation period (35 to 42 days). Significant ($P \leq 0.05$) differences among the different rates of NRTWC were observed at the initial stages of the incubation, however, the variations were minimal after day 14 of the incubation in both soils.

During the first 7 days of incubation, C mineralization ranged from 279 mg/kg (control) to 401 mg/kg (2.8% application rate) after 10 weeks of NRTWC application. The corresponding figures after 20 weeks of application were 258 mg/kg (control) and 668 mg/kg (2.8% application rate) The cumulative C mineralization of both soils are shown in Figure 2. The cumulative total carbon mineralization were significantly different among the four treatments (1%, 1.6%, 2.2% and 2.8%), ranging from 506 to 1072 mg/kg soil for after 10 weeks of treatment application and 506 to 1730 mg/kg soil for 20 weeks after treatment application (Figure 2). The application of NRTWC showed a substantial increase in cumulative carbon mineralization when compared with the control (Figure 2). There were significant ($P \leq 0.05$) differences in carbon mineralization in soils 10 and 20 weeks after treatment application.

Periodic changes in cumulative carbon mineralization after 10 and 20 weeks of different rates of NRTWC application are depicted in Figure 3 (a) and (b) respectively. Significantly ($P \leq 0.05$) higher carbon mineralization was observed in soil amended with NRTWC compared to the control both at 10 and 20 weeks after treatment application. However in the case of cumulative carbon mineralization, no significant ($P \leq 0.05$) difference was observed between the application at rates 1% and 1.6%.

Continuous degradation of tea soils in Sri Lanka is becoming matter of great concern as it causes severe yield reduction. Use of organic amendment to improve organic matter and organic carbon content is an accepted practice as it could ensure the restoration of degraded soils. Furthermore,

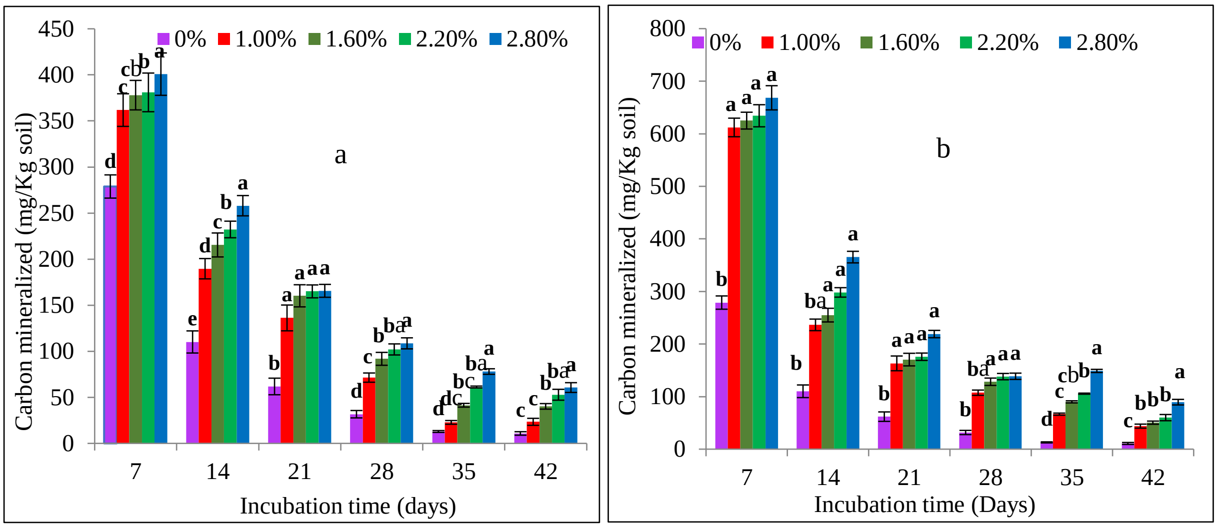

Figure 1: Carbon mineralization after the incubation of soil treated with different rates of NRTWC (a) 10 weeks after treatment application (b) 20 weeks after treatment application. Means with the same letters are not significantly different at p ≤ 0.05

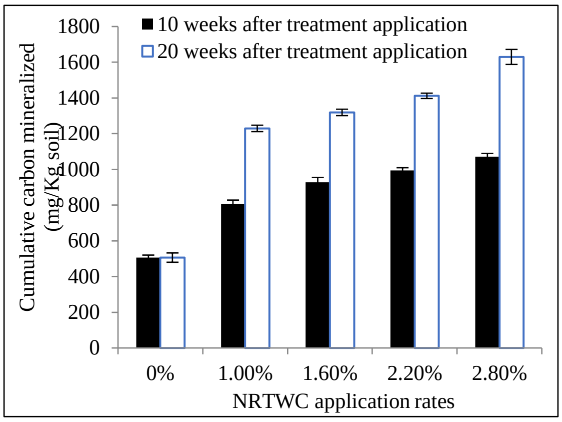

Figure 2: Cumulative carbon mineralization by soil treated with different rates of NRTWC at 10 weeks and 20 weeks after treatment application. Means with the same letters are not significantly different at p ≤ 0.05.

increase in soil organic matter content stimulates the soil microbial biomass, microbial activity and nutrient mineralization (Larkin, 2015; Francioli *et al.*, 2016). Such degradation could be evident in the present experimental soil as it contains low organic matter content, low soil pH (acidic), and high bulk density (Table 1). Therefore, maintaining desirable soil organic matter level in the soil is crucial to enhance soil productivity and soil quality. Pyrolytic carbon black which can be recovered as main product of natural rubber tire pyrolysis is very much similar to biochar in nature (Junqing *et al.*, 2020).

Soil organic carbon mineralization was high during the first week of incubation. Similarly intense increase in carbon mineralization/microbial activity during initial days of the incubation was observed by other researchers (Tsai and Chang, 2019; Luo *et al.*, 2016). This may be due to starting microbial activity following watering to adjust the moisture content to 60% field capacity in treated and control soil before the incubation. NRTWC is a highly porous carbonaceous amendment and its high porosity (macropores) can serve as vital habitats for growth and breeding sites for microorganisms (Pietikäinen *et al.*, 2000). Therefore comparatively higher carbon mineralization could be seen throughout the incubation period when soils treated with NRTWC. Both aerobic and anaerobic bacteria can effectively decompose tire rubber polymers (Stevenson *et al.*, 2008). The microorganisms which are capable of decomposition tire rubber have been isolated by many researchers (Atagana *et al.*, 1999; Bode *et al.*, 2001; Bredberg *et al.*, 2001; Rifaat and Yosery 2004; Khoshgoftarmanesh *et al.*, 2012).

After the first week of incubation, C mineralization showed a decreasing trend during the later part of the incubation which might due to decrease in easily degradable of labile organic carbon for microbial decomposition. Generally, most of the easily available soil organic amendments are found to be readily decomposed and last for a short period of time. NRTWC was produced using pyrolysis process with high temperature with the absence of oxygen. This carbonaceous material contains very high amount of organic carbon (50%) and their aromatic and crystalline structure relatively resistant to microbial decomposition and chemical transformation. Therefore NRTWC could be considered as a stable form of carbon which could remain in the soil for years maintaining the soil organic carbon status. The reduction of carbon mineralization after first week of incubation indicates the reduction in organic matter decomposition. It enhances carbon sequestration and nutrient storage in soil which could therefore minimize the application of inorganic fertilizer (Tsai and Chang, 2019).

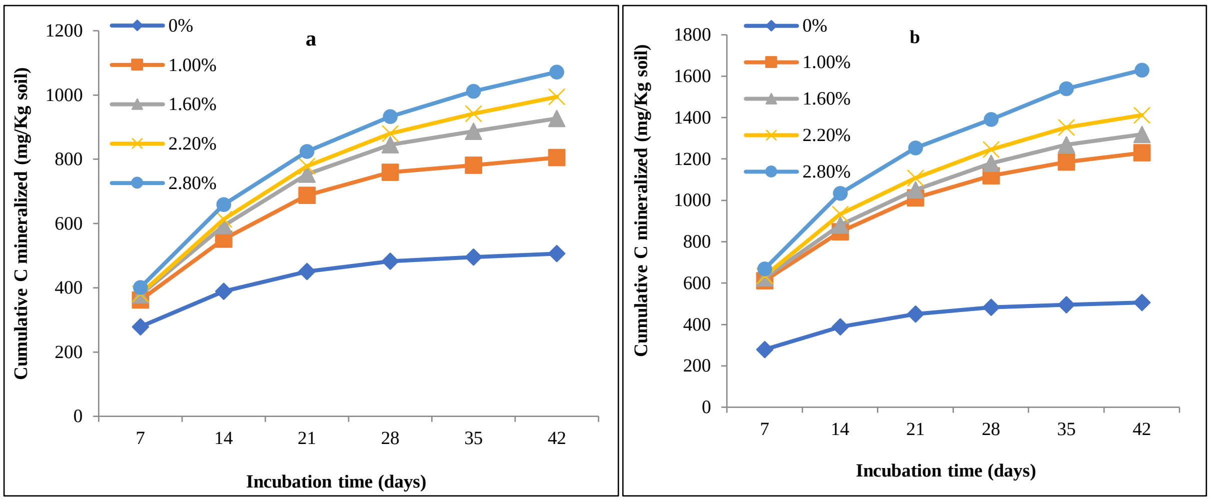

Figure 3: Cumulative carbon mineralization after incubation of soil treated with different rates of NRTWC (a) 10 weeks after treatment application (b) 20 weeks after treatment application. Means with the same letters are not significantly different at $p \leq 0.05$.

As stated by Kuzyakov *et al.* (2009), char begins mineralizing in early stages (during first week), and slow, partially decomposition occurring during the later stage, and finally incorporation into the soil. According to them, addition of char to many low pH agricultural soils can result in major physico-chemical improvements such as microbial activity and availability of soil nutrients. Tea soils have an inhibitory influence on soil microorganisms due to acidity and aluminum toxicity (Karak et al., 2015). As stated by Arafat *et al.* (2020), soil pH in tea estates is significantly lower than in the bulk and 2-year garden soil. According to Xu *et al.* (2014), when char was applied to acidic soil, it enhanced crop yield through improving soil chemical conditions (neutralizing soil pH) and changing the availability of nutrients. It can also have some impacts on the soil microbial community. However, it needs a longer period of time to ameliorate the soil properties and to show a significant impact on plant growth.

CONCLUSIONS

According to the results, rapid carbon mineralization could be observed during the first week of incubation followed by a decline. The cumulative carbon mineralization ranged from 506 to 1072 mg/kg soil for the samples taken at 10 weeks after treatment. The corresponding variation changed from 506 to 1730 mg/kg soil for the samples drawn at 20 weeks after treatment. However, the variations were minimal after second week of the incubation and no changes were observed towards the end of the incubation (35 to 42 days). The cumulative soil carbon mineralization increased rapidly during the early stages, then decreased gradually and finally flattened out towards the end of the incubation. Availability of easily degradable labile organic carbon for microbial decomposition could be the reason for rapid mineralization during the early stages. The gradual reduction of carbon mineralization after the first week of incubation indicates the reduced organic matter decomposition. NRTWC could improve the carbon sequestration and nutrient storage in the soil. Therefore, NRTWC application could increase soil quality while enhancing the carbon sequestration in soil.

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