Research Article

Can the mealybug wilt disease on pineapple be controlled using fertilizers?

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Abstract

Mealybug wilt on pineapple is a threatening problem for the pineapple cultivation worldwide including Sri Lanka. The characteristic symptoms include reddening of leaves, twisting of leaf tips and finally wilting of the whole plant. The disease is caused by a virus known as Pineapple Mealybug Wilt associated Virus (PMWaV) and transmitted by mealybugs. Sri Lankan farmers use many kinds of fertilizers to suppress this disease. The present study attempted to determine whether different fertilizer applications have any effect on managing the above disease while studying the best fertilizer/s to control this disease successfully. Pineapple suckers infected with PMWa virus were planted and six different fertilizer treatments, (Albert's solution, Baur Coconut fertilizer, Baur Pineapple fertilizer, NPK mixture recommended by Department of Agriculture, Green care Liquid fertilizer, Rhizobacteria Bio fertilizer) were applied. Infected plants with no added fertilizer were used as controls. The disease severity was evaluated using five-unit disease index scale. All the plants including the controls were tested for the presence of PMWa virus by Indirect Enzyme Linked Immunosorbent Assay (ELISA). Growth parameters such as number of leaves and leaf length were also measured in all the plants. This study indicated that the disease severity was low on plants treated with Albert's solution throughout the experimental period. The plants treated with Baur Coconut fertilizer showed higher disease severity at the beginning. However, there was a sharp decline in disease severity with time. The plants treated with Baur Coconut fertilizer indicated the lowest virus concentrations followed by those that were treated with Albert's solution. The highest number of leaves were noticed in the plants treated with Baur coconut fertilizer whereas the highest leaf length was observed in the plants treated with Albert's solution revealing that these two fertilizers can control the disease effectively while improving the vegetative growth.

Keywords*: Pineapple, Mealybug Wilt Disease, Fertilizers, PMWaV,*

1. Introduction

Pineapple, *Ananas comosus,* is a semi-perennial monocotyledonous plant with a terminal multiple fruit in the family Bromeliaceae and is widely grown in the tropical and sub-tropical regions across the world (Ulman et al., 1992). In addition to use it as a delicious fruit in day-today consumption, it is also processed into juice, jam, squash and jelly. Fruit core is used to prepare candy and leaves yield 2-3% of white fibers. Dried waste is a valuable cattle feed (Upadhyay et al.,2010). Different pineapple varieties such as Kew pine, Mauritius, Rock pineapple, Red Spanish, Queen, Kew and Mauritius are grown in Sri Lanka (Mankotte and Fernando, 2007) and the country earns substantial foreign returns through exporting pineapple-based products (Central Bank of Sri Lanka, 2016). Although pineapple is a valuable crop, there are so many pests and diseases affecting the growth and development of the inflorescence (Rohrbach and Schmitt, 2003). Some pests that affect pineapple plants are mealybugs, scale insects, thrips, fruit borers, bud moths, midgets, fruit flies, beetles, weevils, and mites.

Mealy bug, (*Dysmicoccus brevipes* and *Dysmicoccus neobrevipes*), is primarily a pest of pineapple, although it does not depend on that single host plant to complete its life cycle. Due to its polyphagous nature, it has been reported on more than 100 plant genera in 53 families (Mau and Kessing, 2007). This pest transmits Pineapple mealybug-associated wilt virus and that makes this a very important pest. The disease is a serious threat to pineapple production island wide. It negatively affects the productivity by reduction in the yield of the cultivation (Jahn et al., 2003). This disease is characterized by severe tip die back, downward curling, reddening and finally wilting of leaves which could lead to total collapse of plants and destroy pineapple cultivations in a short period of time (Rohrbach et al., 1988). Pineapple wilt causes reddening of leaves and subsequent pink coloration. The plant loses rigidity and appears wilted. These symptoms may occur rapidly or slowly. The disease primarily attacks the roots of the plant, which causes the discoloration and leaf symptoms. However, the plants recover and continue growing, but will have reduced weight, leaf size, and root length. (Mau & Kessing, 2007). In Sri Lankan context, farmers may not use any special measures to control this disease, however, they use various kinds of fertilizers to suppress the disease (Dissanayake et al., 2001). Department of Agriculture, Sri Lanka has also recommended various liquid and bio fertilizers that are likely to suppress the pineapple wilt virus. The present study investigated whether different fertilizer applications have any effect on managing mealy bug wilt of pineapple and determined the comparative efficacies of some recommended fertilizers in managing the disease.

This study also examined the developing symptoms of pineapple plants when they are under attack of the virus at different fertilizer regimes and assessed the severity of the disease using Enzyme- Linked Immunosorbent Assay (ELISA).

2. Material and Methods

2.1 Selection of Plants

Symptomatic pineapple (Kew variety) plants were selected randomly from Kosgama area (one of the major pineapple growing areas) and the experimental plots were situated in the premises of Plant Virus Indexing Center (PVIC) in Homagama.

2.2 Different Fertilizer Treatments

One hundred and five pineapple suckers (3-month-old) infected with pineapple mealy bug wilt associated virus were planted in 3 rows. There were 7 blocks in each row and each block consists of 5 pineapple plants (5 replicates) as shown in plate 1. Chick fertilizer was added (100 g/plant) to all plants immediately after planting. The suckers were tested, at the time of planting to ensure that all were uniformly infested with the disease using ELISA.

Plate 1: Experimental plot showing distance between pineapple plants and rows (Distance between two pineapple plants in a row (A) – 30 cm; Distance between two rows (B) – 150 cm)

One and a half months after planting, five different fertilizer applications were given as described below. The quantity of fertilizers was decided according to the Handbook of pineapple cultivation, Department of agriculture, Sri Lanka (2007).

Fertilizer applications were continued every two months. Infected plants with no fertilizer added were used as controls. One treatment was given for one block and the treatments were randomized between blocks as shown in Table 1. It was assumed that there was no mixing of different fertilizers.

Treatments

- BCO Baur coconut fertilizer (35g/plant)
- BPA Baur pineapple fertilizer (35g/plant)
- GCR Green care liquid fertilizer (30 ml/plant)
- RHZ Rhizobacteria bio fertilizer (40ml/plant)
- NPK NPK mixture recommended by Department of agriculture Sri Lanka (35g/plant)
- ABS Albert's solution (30 ml/plant)
- CON Infected plants with no fertilizer added (Control)

Liquid fertilizers were prepared according to the instructions given on the bottle/packet.

Each treatment was given for 15 replicate plants as shown in Table 1. All plants were labeled according to the treatment given. Liquid fertilizer was added monthly while solid and bio fertilizers were added at two-month intervals. Each plant was subjected to visual observation of symptoms every month and the mean disease index was calculated. Enzyme- Linked Immunosorbent Assay (ELISA) was carried out in two-month intervals to determine the virus concentration.

Table 1: Treatment Plan

	Block1	Block2	Block3	Block4	Block5	Block6	Block7
R1	CON	NPK	GCR	BPA	RHZ	BCO	ABS
R2	RHZ	BCO	BPA	GCR	CON	ABS	NPK
R3	NPK	RHZ	GCR	BPA	ABS	BCO	CON

R- Rows Each block consists of five plants

- ABS Albert's solution
- BCO Baur coconut fertilizer
- BPA Baur pineapple fertilizer
- GCR Green care liquid fertilizer
- NPK NPK mixture recommended by department of agriculture Sri Lanka
- RHZ Rhizobacteria bio fertilizer
- CON Infected plants with no fertilizer added

2.3 Visual Observations, Enzyme - Linked Immunosorbent Assay (ELISA) and growth parameters

2.3.1 Visual observations

Visual symptoms were recorded in all pineapple plants in the experimental plot and rating was done according to the severity of the symptoms. Total marks and total disease index were computed for each plant using a formula. The disease symptoms considered were reddening of leaves (A), tip wilting (B), leaf twisting (C) and wilting of whole plant (D). Symptom expression was scored in 0-5 scale. As a standard, '0' was given to the plants in which the leaves were apparently healthy in appearance.'5' was given to the plants in which leaves were severely affected and showing clearly distinct viral symptoms. Numbers that lie between were also given marks according to the severity of the symptoms. This visual assessment was done monthly.

Reddening of leaves (A)	0-5
Tip wilting (B)	0-5
Leaf twisting (C)	0-5
Wilting whole plant (D)	0-5
Total	0-20

Total disease Index (TDI) = A+B+C+D

Mean disease index $= \frac{Total\ disease\ index}{Total\ No\ of plants}$

2.3.2 Enzyme - Linked Immunosorbent Assay (ELISA)

Virus concentration was measured by indirect ELISA using locally produced polyclonal antiserum at two-month intervals. ELISA was carried out using two randomly selected plants from each treatment. ELISA was also done for non-infected healthy pineapple plant samples obtained from tissue culture laboratory and infected plants obtained from disease house at Plant Virus Indexing Centre to compare the ELISA values of healthy plants with that of infected plants to study the severity of infection.

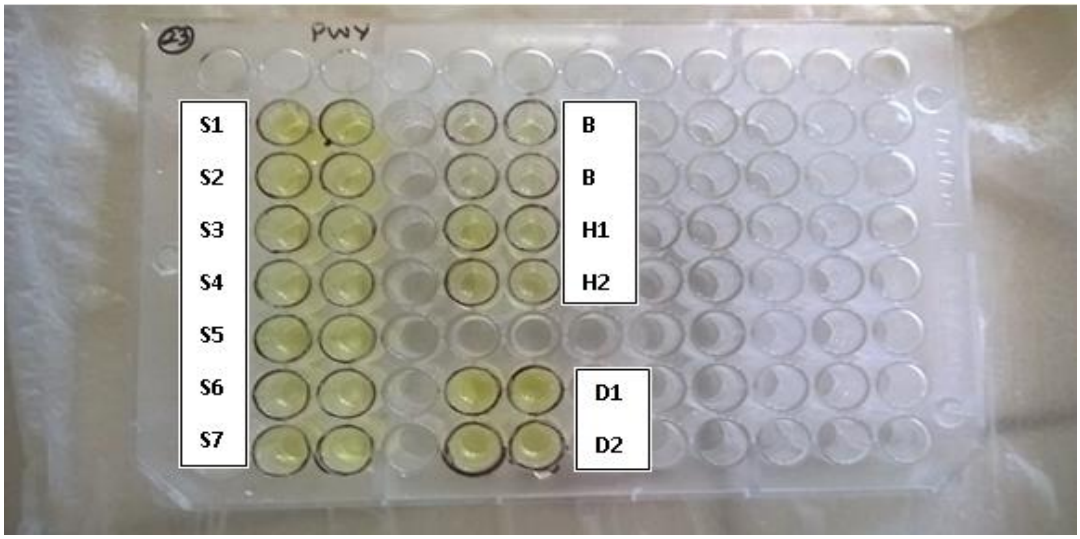

Plate 2: ELISA plate after 1-hour incubation period. This plate was used to calculate ELISA values using a computer programmable ELISA plate reader

S1-Baur Pineapple Fertilizer
S2-Baur coconut Fertilizer
S3-Rhizobacteria bio fertilizer
S4-Albert Solution
S6-Green care
S7-Control

B-Buffer solution
H1- Healthy pineapple 1
H2- Healthy pineapple 2
D1-Infected pineapple plant
D2- Infected pineapple plant

2.3.3 Measurement of growth parameters

Initial leaf length measurements were taken four months after giving different fertilizer treatments. Number of leaves per plant was also recorded at the same time. Final measurements were taken at the end of the experiment period when plants were nine months old. Finally, the average length of leaves per plant and average number of leaves per plant were calculated. Fifteen replicate plants per treatment were used for leaf length measurements and to count the number of leaves.

2.3.4 Data analysis

The results were analyzed statistically by 3-way ANOVA using SPSS version 16 (2008) package.

3. Results and Discussion

3.1 Visual Observations

Table 2: Effect of different fertilizers on mean disease index

Fertilizer	April	June	July	Aug	Sept	Oct	Nov	Dec
NPK Mixture	1.93	3.46	4.47	4.80	4.50	4.20	4.37	4.40
Baur Pineapple Fertilizer	2.20	4.60	5.40	3.93	4.21	4.00	4.33	4.20
Baur Coconut Fertilizer	2.13	4.13	4.13	2.53	2.45	2.80	2.93	2.83
Green Care Liquid Fertilizer	2.00	4.53	6.27	3.40	4.22	4.36	4.26	4.39
Albert Solution	1.60	1.80	2.00	2.20	2.20	2.12	2.32	2.33
Rhizobacteria Bio Fertilizer	2.33	3.60	6.80	3.14	4.55	5.12	4.34	5.93
Control	1.93	5.06	4.60	3.86	4.40	4.20	4.45	4.48

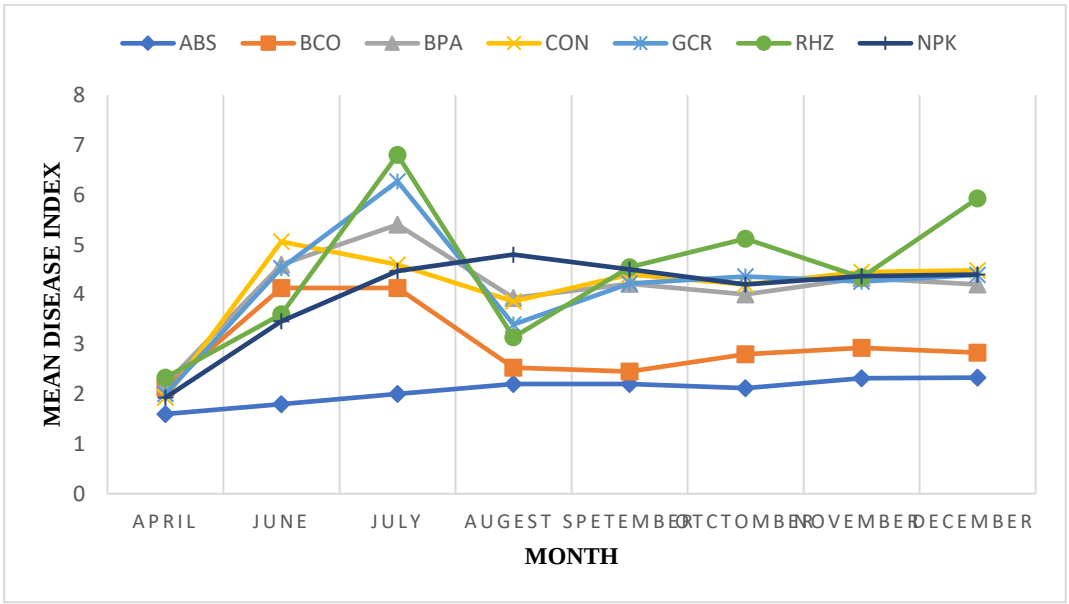

Figure 1: Effect of different fertilizers on mean disease index

The disease index prepared to evaluate the disease severity showed that all plants had equal symptoms at the time of commencement of different fertilizer treatments.

Plants treated with Rhizobacteria bio fertilizer and Green care liquid fertilizer showed the highest disease severity at the beginning when compared to control plants and plants treated with other fertilizers. Although disease severity became less in these plants with time, it was higher than in control plants. The plants treated with Baur coconut fertilizer showed a higher disease severity at the beginning. However, it is interesting to note that there was a sharp decrease in disease severity with time when compared to controls and plants treated with Baur pineapple fertilizer, Rhizobacteria bio fertilizer, Green care liquid fertilizer and NPK mixture. The disease severity was very low in plants treated with Albert's solution throughout the experimental period (Figure 1).

Experimental results of the present study indicated that the Albert's solution and Baur coconut fertilizer were most effective fertilizers to control the mealybug wilt of pineapple. Albert's solution contains a high amount of Ca in addition to N, P, K and many of the micronutrients. Baur coconut fertilizer has a high amount of N and K when compared to other fertilizers used in this study.

Drowner (2000) has reported that mineral nutrition can affect two basic plant resistance mechanisms in plants. These mechanisms include formation of mechanical barriers such as cell wall strengthening and synthesis of defense compounds that protect against pathogens (Spann and Schumann, 2010). He has also reported that deficiencies of calcium and potassium can interrupt either of these defense mechanisms.

It has been found that calcium affects the susceptibility to diseases in two ways. First, Ca is important for the stability and function of plant membranes and when there is a Ca deficiency, there is membrane leakage of low molecular weight compounds such as sugars and amino acids from the cytoplasm to the apoplast, which stimulates the infection by the pathogens (Marscner, 1995). Second, Calcium is an important component of the cell wall structure as calcium polygalacturonates are required in the middle lamella for cell wall stability. Therefore, it can be assumed that the high amount of Ca present in Albert's solution has aided in managing the mealybug wilt of pineapple in the present study, due to the above-mentioned reasons.

Huber and Graham (1999) have reported that potassium decreases the susceptibility of host plants up to the optimal level for growth. It has been found that the high susceptibility of the K-deficient plants to parasitic disease is due to the metabolic functions of K in plant physiology (Dordas, 2008). Under K deficiency, synthesis of high molecular weight compounds such as proteins, starch and cellulose are impaired and there is accumulation of low molecular weight organic compounds which stimulate the infection by pathogens. In addition, Dordas (2008) has reported that K may promote the development of thicker outer walls in epidermal cells thus preventing disease attack. It also has been reported that K can influence plant

metabolism, as K-deficient plants have impaired protein synthesis and simple nitrogen compounds such as amides which are used by invading plant pathogens (Dordas, 2008). Therefore, the reduced disease severity of the plants treated with Baur coconut fertilizer in this study can be attributed to the presence of high amount of potassium.

3.2 Enzyme - Linked Immunosorbent Assay (ELISA)

ELISA can be very effectively applied to detect and assay of plant viruses. There are many variations of the basic procedure of ELISA and indirect ELISA method was used to assay PMWaV in pineapple plant samples in the present study.

Table 3: Values of Enzyme - Linked Immunosorbent Assay

Fertilizer	April	June	August	October	December
Albert Solution	0.026	0.031	0.055	0.056	0.037
Baur Coconut	0.045	0.033	0.018	0.023	0.022
Baur Pineapple	0.066	0.038	0.060	0.053	0.046
Control	0.034	0.054	0.169	0.047	0.055
Green Care	0.037	0.034	0.077	0.049	0.046
NPK	0.029	0.047	0.078	0.024	0.029
Rhizobacteria	0.032	0.049	0.059	0.055	0.056
Healthy Samples	0.022	0.020	0.003	0.016	0.020
Diseased Samples	0.085	0.081	0.137	0.068	0.053

Table 4: Mean values of Enzyme- Linked Immunosorbent Assay

Treatment	Mean
Albert's solution	0.04100 (0.013802)
Baur Coconut	0.02700 (0.012193)
Baur Pineapple	0.05160 (0.012818)

Control	0.07180 (0.054979)
Green Care	0.04860 (0.017038)
Rhizobacteria	0.0502 (0.10803)
NPK	0.05133 (0.024786)

The results of Enzyme - Linked Immunosorbent Assay revealed that plants treated with Baur coconut fertilizer have the lowest viral concentrations (Figure 2). As indicated in Table 3, it remained low throughout the experimental period, and this was followed by the plants treated with Albert's solution. ELISA values obtained for plants treated with Baur pineapple fertilizer, Rhizobacteria bio fertilizer, Green care liquid fertilizer and NPK mixture were higher than that of plants treated with Albert's solution and Baur coconut fertilizer and lower than the control plants.

Although, Enzyme - Linked Immunosorbent Assay revealed that the virus concentrations were less in plants treated with Baur coconut fertilizer, the disease severity of the plants treated with Baur coconut fertilizer was higher than that of plants treated with Albert's solution. This may be due to the use of only a few randomly selected pineapple samples for Enzyme - Linked Immunosorbent Assay as this technique requires sophisticated equipment as well as expensive chemicals.

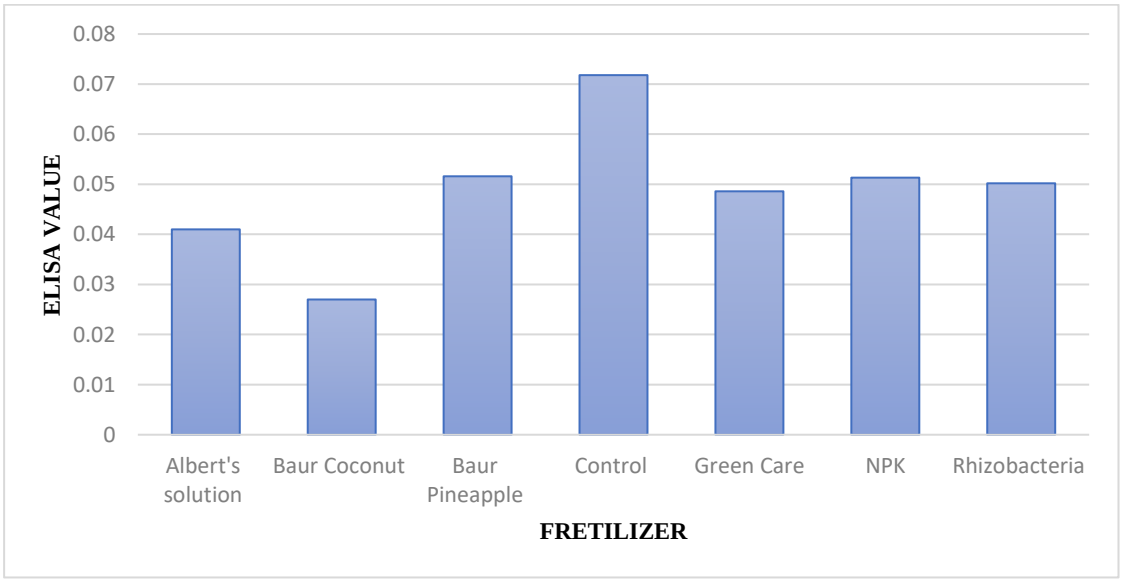

Figure 2: Effect of different fertilizers on values of Enzyme - Linked Immunosorbent Assay

3.3 Growth Parameters

Table 5: Effect of different fertilizers on number of leaves and length of leaves

Treatment	Mean Number of leaves		Mean leaf length (cm)	
	June	December	June	December
Albert solution	23	25	46.84	50.60
Baur Coconut	23	29	44	53.22
Baur Pineapple	18	22	44.78	46.34
Control	17	22	41.34	43.89
Green care	19	21	39.38	41.56
NPK	17	24	44.67	48.12
Rhizobacteria	16	18	57.3	57.3

Table 6: Effects of different fertilizers on mean number of leaves and mean length of leaves

Treatment	Number of leaves June	Leaf length (cm) June	Number of leaves December	Leaf length (cm) December
Albert solution	28.87^{c} (2.36)	58.57^{d} (5.48)	32.13^{d} (2.48)	58.99^{c} (6.92)
Baur Coconut	29.93^{b} (4.13)	57.75^{c} (4.43)	36.73^{e} (5.37)	61.03^{b} (4.23)
Baur Pineapple	17.93^{a} (2.60)	44.48^{a} (7.60)	23.53^{c} (1.55)	44.79^{a} (1.47)
Control	17.20^{a} (2.78)	39.20^{a} (8.12)	20.87^{a} (1.73)	42.66^{a} (7.73)
Green care	17.07^{a} (2.92)	41.91^{a} (7.51)	21.33^{a} (3.06)	43.23^{a} (4.84)
NPK	18.47^{a} (2.10)	45.64^{a} (7.63)	24.53^{b} (2.59)	45.94^{a} (2.62)
Rhizobacteria	17.20^{a} (1.86)	45.87^{b} (10.99)	20.20^{a} (2.24)	44.57^{a} (11.52)

Different letters along the Column indicate the mean was significantly different at P<=0.05

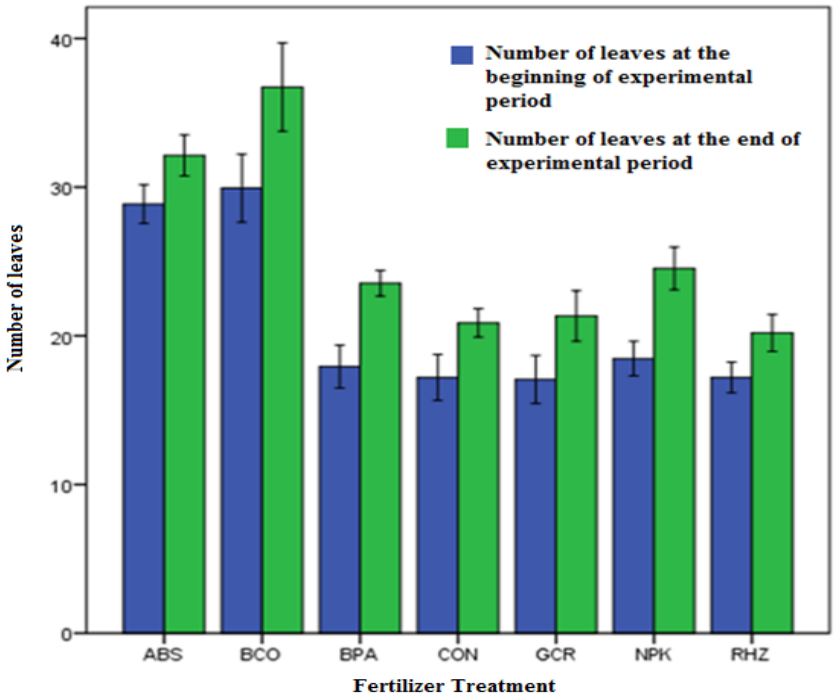

Figure 3: Effect of fertilizers on number of leaves

The figure 3 revealed that the mean number of leaves has increased with time in all the treatments during the experimental period. However, this increase was more prominent in the plants treated with the Albert's solution and Baur's coconut fertilizer. This indicated that these two fertilizers promote vegetative growth more in pineapple. As shown by figure 4, mean number of leaves has increased with time during the period under consideration with all the treatments except the treatment with Rhizobacteria, in which there is a slight decrease indicated but this was not significant. In this instance also the albert's solution and the Baur's coconut fertilizer have exhibited that they were more effective for the vegetative growth.

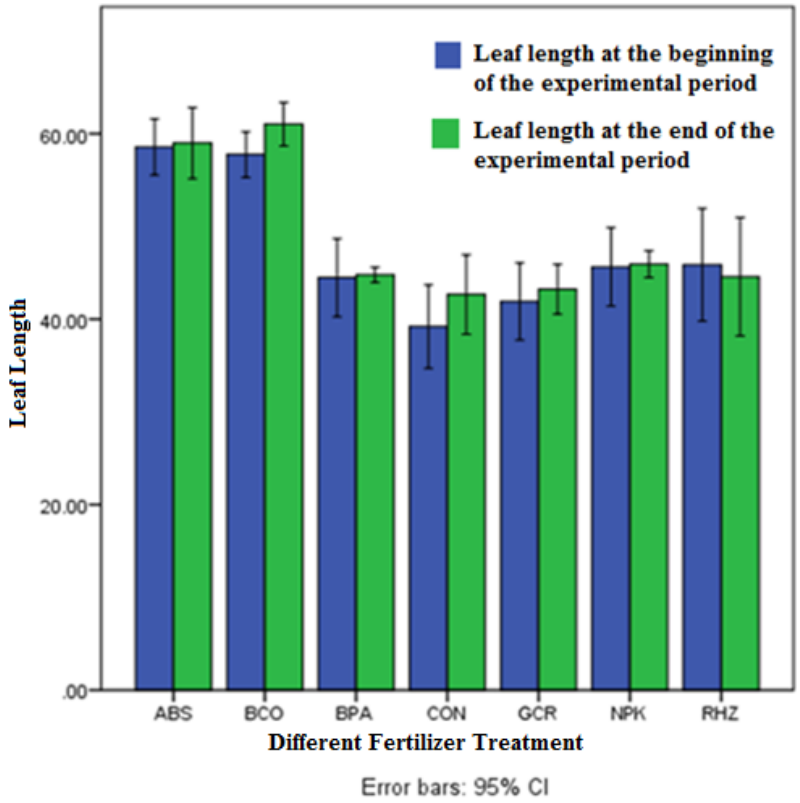

Figure 4: Effect of fertilizers on mean leaf length

In addition, this study has also shown that the plants treated with Baur coconut fertilizer have more vigorous growth (higher number of leaves) when compared to other treatments. This may be due to the presence of high amounts of N and K in Baur coconut fertilizer, both of which are important nutrients for plant growth.

According to National agricultural research organization (NARO Annual report, 2003), pineapple mealybug wilt disease is a widespread and devastating disease in many pineapple growing areas of the world. Pineapple wilt disease is also a common occurrence in pineapple cultivations in Sri Lanka where the pineapple is grown as a commercial crop.

Pineapple is the only known plant host of PMWa virus which is the causative agent of the disease. Therefore, new infections arise from infected planting material or by mealybugs acquiring the virus from infected pineapple plants and moving to other pineapple plants.

Eradication of the virus by planting PMWaV-free plant material is a possible management strategy. It has been shown that this virus can be eliminated either by apical or meristematic bud propagation (Sether et al., 2001) or heat treatment of

infected planting material (Dassanayaka and Perera, 2001). Sometimes plant material used for planting new crops is dipped in an insecticide effective against mealy bugs prior to being planted.

However, integrative plant nutrition is an essential component in sustainable agriculture, because in most cases it is more cost effective and also environmentally friendly to control plant disease with the adequate amount of nutrients and with no pesticides.

In Sri Lanka, farmers do not use any special treatments to control the pineapple mealybug wilt disease but use various kinds of fertilizers to manage the disease.

Much research has been carried out to find the effects of fertilizers on various plant diseases. Parthasarathy (2015) has reported that an adequate mineral nutrition can exert a considerable influence on disease development. It has also been reported that fertilizer applications can increase or decrease the development of diseases caused by different pathogens and the mechanisms responsible are complex including effects of nutrients on plant growth, plant resistance mechanisms and direct effects on the pathogen.

Since plants treated with both Albert's solution and Baur coconut fertilizer show less disease severity and more vegetative growth when compared to other fertilizers, it can be recommended to apply Albert's solution and Baur coconut fertilizer alternately to control the Mealybug Wilt of Pineapple more successfully.

However, further studies are necessary to confirm this evidence and also how these fertilizers affect the fruiting of pineapple which is most important to the cultivators, needs to be considered.

4. Conclusions

To control Mealybug Wilt of pineapple fertilizers such as Albert's solution and Baur's coconut fertilizer can be successfully applied because these improve the vegetative growth. Albert's solution is more effective in reducing disease severity, while Baur's coconut fertilizer stimulates vigorous growth of infected pineapple plants, but not effective in reducing most of the viral symptoms.

However, further studies are recommended to confirm these findings and also to detect how these two fertilizers would affect the yield and the quality of the product.

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