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Decomposition of Sugarcane Trash by Selected Microbes and their Biofilms: A Laboratory Investigation

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ABSTRACT

Natural decomposition of sugarcane trash is slow, taking more than three months, and hence, it becomes a problem with inter-cultivation and restricts the growth of the ratoon of the sugarcane crop. Trash blanketing is a common management practice carried out but does not solve the above problems. This study investigates the potential of using selected microbial combinations, including fungal-bacterial biofilms (FBB), for rapid decomposition of sugarcane trash. Two studies were carried out at laboratory level, namely, to identify the sugarcane trash decomposition process and then to evaluate selected microbial combinations in enhancing sugarcane trash decomposition. In the first study, sugarcane trash samples were placed in 24 wells of a tissue culture plate separately, and evaluated the surface functional groups of organic compounds by Fourier transform infrared (FTIR) spectroscopy. In the second study, 24 treatments in a completely randomised design were used to identify selected microbial combinations that could be effective in sugarcane trash decomposition. When considering the results of the first study, there was a positive correlation between weight loss and FTIR peak degradation of organic molecular functional groups, particularly O-H of the carboxylic group, C-H of the aromatic methyl group, and Si-O of the cuticle wax layer. The results of the second study showed that urea being a chemical treatment, was significantly effective in reducing the C/N ratio of decomposed trash. In the microbial treatments, bacterium B2 (yet to be identified) was effective on trash fragmentation, and the FBB coded as F1F2B1B2 was effective on both trash fragmentation and reducing its C/N ratio. The combination should therefore be tested for trash decomposition in the long run of the sugarcane crop cycle under field conditions.

INTRODUCTION

Sugarcane trash, an important source potentially for soil improvement, is left on the field following the harvest of sugarcane stalks. However, its slow decomposition process has become a problem with inter-cultivation as it takes more than three months. This may be due to the chemical composition of trash, which includes organic compounds, cellulose, hemicellulose, and lignin in percentages 45.1, 25.6, and 12.7, respectively, along with other minor components such as inorganic materials, ash, silicon, chlorine, and metals (Woytiuk, 2006).

Effective management of trash leads to greater profits for the sugarcane grower. Trash blanketing is a common management practice that improves the soil properties like water holding capacity, organic matter, crumb structures, and total exchange capacity (Thompson, 1966). But there are drawbacks of trash blanketing where it can restrict the growth of ratoon crops by causing temporal nutrient immobilisation and interfering with inter-cultivation practices.

The decomposition of organic matter is the process by which they are converted into smaller and simpler compounds. It is a biological process carried out by macro and microorganisms (Kuers and

Simmons, 2006). Following initial decomposition, humification takes place, resulting in the formation of humus. During this process, initially degradable carbon sources start to decompose, and then more resistant compounds like lignin degrade into smaller units by the action of extracellular enzymes (Varadachari and Ghosh, 1984). Microorganisms play a major and important role in the decomposition process. Fungi can decompose lignocelluloses, cellulose, and hemicellulose (Tuomela *et al*., 2000). Further, Basidiomycotina is effective on lignin (Eriksson *et al*., 1990). Bacteria contribute by consuming the small molecular weight intermediate compounds which are produced by fungi (Vicuna, 1988 and Ruttimann *et al*., 1991). A study done by Dixon (2013) highlights that both fungi and bacteria are important in plant litter decomposition as they fulfill their nutritional sugar requirement by breaking down plant cell walls. The same study shows that fungi use degradable enzymes called cellulases, which uniformly dissolve the wall from the innermost side. Bacteria use a multiple enzyme complex called cellulosomes, and they start digesting the cell walls away from the middle lamella. Therefore, this clearly shows that the decomposition process could be accelerated when fungi and bacteria are together, like in FBBs, rather than in isolation (Seneviratne *et a*l., 2008).

Biofilms in soils are complex multicellular communities comprising mainly fungi and bacteria, where the bacteria may adhere to the biotic surface of the fungus (Seneviratne et al., 2008). Biofilms adhere to the plant roots of crops and play an important role in improving agricultural production by cycling the nutrients and also by controlling pests and diseases. Therefore, the present study investigates the potential of using selected microbial combinations, including FBBs, for the rapid decomposition of sugarcane trash.

MATERIALS AND METHODS

The investigation was carried out in two studies, 1) to identify the sugarcane trash decomposition process and then, 2) to evaluate selected microbial combinations in enhancing sugarcane trash decomposition.

Study 1: Identification of sugarcane trash decomposition process

Sugarcane-growing soil from Uda Walawe was used, and it was air-dried and sieved through a 0.5 mm sieve before the experiment. Fresh sugarcane trash pieces (1 cm x 1 cm) were cut from the middle part of the trash in between the edge and the midrib, and twenty-four-well tissue culture plates were used for the experiment. Soil (0.5 g) was weighed and put into each well of the tissue culture plates. A synthetic mesh was cut and placed above the soil layer (Figure 1). The initial weights of fresh trash pieces were recorded, and then they were placed on the synthetic mesh and kept in the incubator at 37 °C. Another 3 - 4 fresh trash pieces were taken, and their initial weights and oven-dried weights were recorded to calculate the moisture factor for calculating the dry weights of the trash pieces.

Figure 1: The layout of a tissue culture plate after the establishment of the 1st study

Analysis

Trash samples were ground into powder by using a mortar and pestle. Potassium bromide (KBr) pellets were prepared with the weight ratio of 1:100 of sample:KBr pellets were subjected to spectral analysis

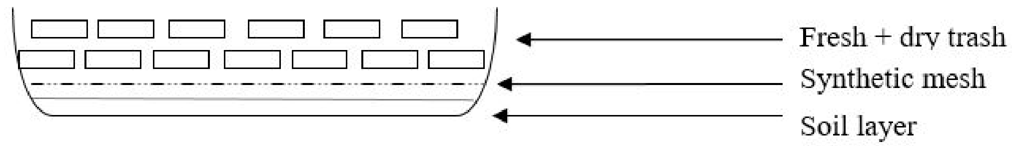

Figure 2: A cross section of Petri plates after establishment of the 2nd study

of Fourier transform infrared (FTIR) spectrophotometer for evaluation of surface functional groups of organic compounds.

Study 2: Evaluation of selected microbial combinations in enhancing sugarcane trash decomposition

Disposable sterilized plastic petri plates were used for the experiment. One gram of 2 mm sieved soil was put into the petri plates, and a synthetic mesh was placed on it. Both fresh and dry trash pieces were cut into 3 cm x 1 cm pieces (Figure 2). According to the fresh/dry trash weight ratio at the harvesting of mature sugarcane in the field, 1.4 g of fresh trash and 1 g of dry trash were measured separately and laid above the synthetic mesh.

Experimental design and treatments

The plates were arranged in a completely randomized design (CRD) with 24 treatments and 3 replicates under laboratory conditions. was used with DMRT mean separation method at 5% probability level for comparing the effects of the treatment on the crop parameters and the moisture parameters.

Microbial treatment combinations

Fungi and bacteria previously isolated from sugarcane fresh and dry trash and their combinations (FBBs) were used for this study. The microbial cultures included two fungi (F1 = Mucor spp and F2 = un-identified) and two bacteria (B1 and B2 both un-identified). The microbial combinations included three FBBs and two mixed cultures. The microbial treatments were also separately treated with a known dilution of molasses (Table 1).

Table 1. Microbial treatments used in the 2nd study

Treatment no.	Treatments (monocultures, biofilms, and mixed cultures)
1	Fungi-1 (F1)
2	Fungi-2 (F2)
3	Bacteria-1 (B1)
4	Bacteria-2 (B2)
5	F1F2B1B2
6	F1B1B2
7	F2B1B2
8	F1F2
9	B1B2
10	100% urea (basal dressing of 50 kg/ha)
11	50% of urea from the basal dressing
12	Control
Treatment no	Treatments mixed with molasses
13	F1 + Molasses
14	F2 + Molasses
15	B1 + Molasses
16	B2 + Molasses
17	F1F2B1B2 + Molasses
18	F1B1B2 + Molasses
19	F2B1B2 + Molasses
20	F1F2 + Molasses
21	B1B2 + Molasses
22	100% Urea + Molasses
23	50% Urea + Molasses
24	Molasses

Treatment application

Microbial monocultures, mixed cultures, and FBBs were applied at the rate of 50 ml per 0.1 ha. Urea was added to the rate of 50 kg/ha for 100 % urea-added treatments and exactly half for the 50 % urea application. One gram of molasses was diluted 1000 times, and 2 ml was applied for appropriate treatments.

Data analysis

Data were analysed by ANOVA procedure using SAS® software, and mean separation was done by Tukey's test. All the interpretations were made at 95 % probability ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Study no 1: Identification of the sugarcane trash decomposition process.

Dry mass of trash during decomposition reduced with time, as given in Figure 3. The highest sugarcane trash decomposition occurred during the initial three weeks, and out of that, the third week was prominent. This can be attributed to the higher microbial activity coupled with higher nutrient content. The activity declined with the depletion of nutrients. The weekly fluctuation of the decomposition is a result of microbial succession.

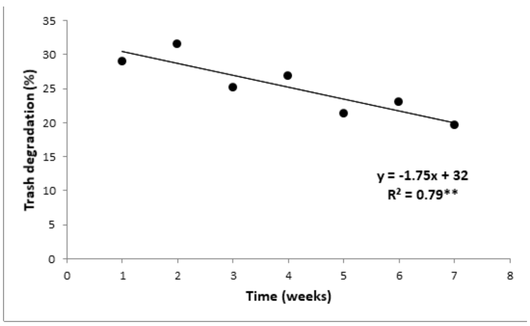

Figure 3: Sugarcane trash decomposition with time

Correlation between percentage dry weight loss and FTIR peak degradation during sugarcane trash decomposition showed fairly significant relations for several peak numbers (Table 2). The Peak at 3410 cm^{-1} resulted in the highest correlation, followed by peaks at 2918, 1046, and 1383 cm^{-1}. These peaks reflected the presence of O-H, C-H, Si-O, and CH_3-R bonds (Table 3), which are generally present in biochemical compounds such as carbohydrates (cellulose, hemicellulose), cuticle wax layer of grasses and amino acids of proteins, respectively.

Table 2: Pearson correlation coefficients between percentage dry weight loss and FTIR peak degradation during sugarcane trash litter degradation from 0 to 8 weeks

Peak number (cm^{-1})	Pearson Correlation Coefficients(r)	Probability (P)
3410	0.7652	0.0269
2918	0.6569	0.0768
2850	0.2520	0.5471
2360	0.3988	0.3277
1716	-0.1018	0.8104
1735	0.3890	0.3409
1637	0.2241	0.5937
1515	0.1747	0.679
1540	-0.1018	0.8104
1557	-0.0104	0.9804
1458	-0.0075	0.986
1421	-0.0243	0.9545
1383	0.4948	0.2126
1246	0.4857	0.2224
1160	0.1335	0.7527
1073	-0.0466	0.9128
1046	0.6143	0.1052
897	0.1795	0.6707
799	0.2010	0.6332
668	0.4482	0.2653
561	0.4795	0.2292
515	-0.0788	0.8528
470	0.4229	0.2966

Table 3: FTIR peak numbers and respective functional groups of organic molecules (Smidt *et al*., 2011)

Peak Number (cm^{-1})	Functional group
3410	O-H stretching in hydroxyl group
2918	C-H stretching in anti-symmetric aliphatic methylene group
2850	C-H stretching in symmetric aliphatic methylene group
1735	C=O stretching in symmetric aldehyde, ketone, carboxylic acid, esters
1515	Aromatic skeletal lignin, lignocellulosic materials
1383	CH_3-R stretch in nitromethane
1246	C-O-C stretching vibration in esters C-N stretching vibration in amide-iii
1046	Si-O stretching vibration Si-O-Si stretching vibration in Silica
668	S=O bending vibration in sulphate
561	P=O stretching of phosphate
470	NH^+ torsion, COO^- stretching

There were positive correlations between total weight loss during 0 to 8 weeks and FTIR peak degradation at peak numbers 3410 cm^{-1}, 2918 cm^{-1}, and 1046 cm^{-1} during the 3rd, 4th, 5th, and 7th weeks (Figure 4). Thus, total weight loss was reflected by total peak degradation of the above-mentioned peaks, meaning that breakage of litter surface functional groups of biochemical compounds can be used to predict long-term trash decomposition. The major part of weight loss occurred during the 3rd, 4th, and 5th weeks.

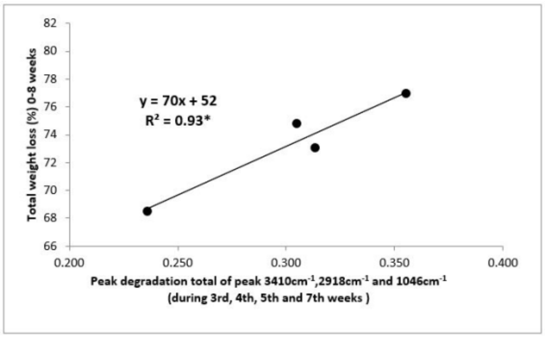

Figure 4: Relationship between total FTIR peak degradation and total weight loss of sugarcane trash.

Study no 2: Evaluation of biofilm activity on enhancing sugarcane trash decomposition.

The results of trash fragmentation percentage, carbon to nitrogen (C/N) ratio, and weight loss were elaborated on in detail

.

Trash fragmentation percentage

The highest trash fragmentation percentage was shown in the 50% urea + Molasses treatment. However, they were not significantly different from other treatments at a 5% probability level. When considering microbial treatments alone, the highest trash fragmentation percentages were recorded with B2, followed by F1F2B1B2. With molasses, B2 showed the highest fragmentation, followed by F2B1B2. It was observed that microbial monocultures and FBBs' had greater ability in trash fragmentation (Figure 5). Generally, the FBBs are more effective in litter decomposition as they have better growth and colonization abilities compared to their monocultures (Seneviratne *et al.*, 2008).

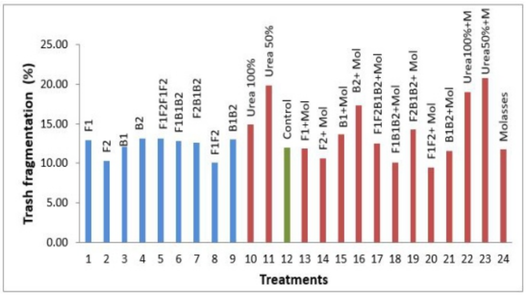

Figure 5. The trash fragmentation percentage of the 24 treatments applied to sugarcane trash, observed after one month.

Carbon to nitrogen ratio

During trash decomposition, the C/N ratio decreased as carbon-containing complex molecules were broken down into simpler molecules. If decomposition results in a lower C/N ratio, it indicates increased decomposition. The lowest C/N ratio was observed in 100% urea, followed by 50% urea (Fig. 6). In the microbial treatments, F1F2B1B2 showed the lowest C/N ratio, followed by F1B1B2.

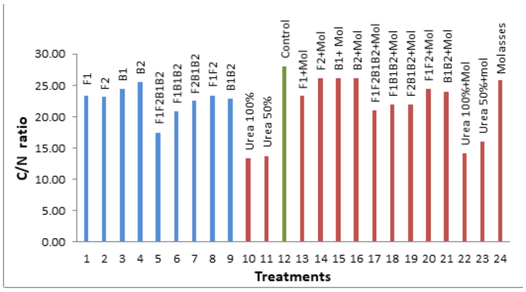

Figure 6: C/N ratio of sugarcane trash in 24 treatments after one month.

Trash decomposition is greatly affected by both endogenous and exogenous N. Endogenous N is the N in the trash, and exogenous N is the N available in the surroundings (Berg and McClaugherty, 2008). Also, it is affected by the type of N (mineral or organic) added, and the amounts of N applied (Fog, 1988). Biofilms are a natural way to get the benefits of synthetic fertilizers without risking the quality of soil health. Diazotrophic bacteria in a FBB help in fixing atmospheric N, thus reducing the C/N ratio (Seneviratne *et al.*, 2008).

According to Figure 7, there is an inverse relationship between the C/N ratio and weight loss which means an increment in weight loss of sugarcane trash with the reduction in the C/N ratio.

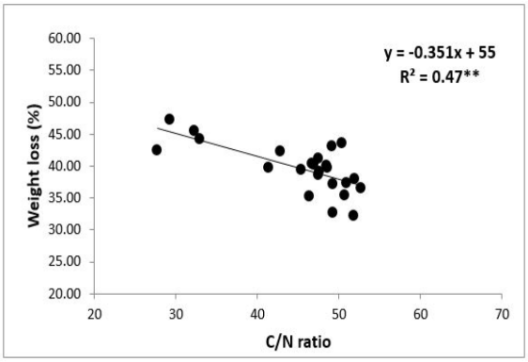

Figure 7: The relationship between C/N ratio and weight loss percentage of decomposed sugarcane trash in 24 treatments after one month.

Results in identifying the sugarcane trash decomposition process conclude that there is a positive correlation between weight loss and FTIR peak degradation of organic molecular functional groups, particularly O-H of the carboxylic group, C-H of the aromatic methyl group, and Si-O of cuticle wax layer. Therefore, sugarcane trash weight loss after two months can be predicted by the breakage of functional groups of organic molecules at 3rd week by using the FTIR peak degradation.

CONCLUSION

In the study to evaluate selected microbial combinations in enhancing sugarcane trash decomposition, the microbial combinations, including FBBs showed promising results even though the urea treatments on C/N ratio were significantly higher than that of the other treatments under laboratory conditions. If more effective N_2 fixing bacteria were identified and incorporated into the microbial combinations as FBBs, it could be more effective on trash decomposition in the long run of the sugarcane crop cycle.

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A New Pre-emergent herbicide formulation for weed control in sugarcane

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ABSTRACT

Continued application of the same chemicals as herbicides could develop resistance of weeds to the herbicides. Hence, screening of newly developed herbicide formulations is essential to find out more effective herbicides for controlling weeds in sugarcane at different growth stages of the crop and the weeds. An experiment was conducted to find out the efficacy of a new herbicide formulation, Diuron 46.8 % + Hexazinone 13.2 % (DI+HEX), in controlling weeds in sugarcane as a pre-emergent and early-post emergent application. The identification of the effective dosage of the chemical in the observational field experiment and the replicated field experiment of controlling weeds were done at the Sugarcane Research Institute, Uda Walawe in 2016/17. A pilot project of application of effective dosage of DI+HEX in 0.5 ac of sugarcane field was conducted in a farmer's field at Lanka Sugar Company (Pvt) Ltd, Sevanagala. The effect of DI+HEX on weed knock-down, residual activity, and phytotoxicity on sugarcane was evaluated in all experiments. It was identified that the application of a new herbicide formulation, DI+HEX, at the rate of 3.0 kg/ha is effective and better than the application of recommended herbicide, Diuron 80 WP, at the rate of 3.5 kg/ha in controlling grass and broadleaved weeds at the pre-emergent stage. However, DI+HEX is not effective in controlling sedges, particularly Cyperus rotundus. Therefore, the application of DI+HEX at the rate of 3.0 kg/ha mixed with 400 litres of water at the pre-emergent stage is recommended to control grass and broadleaved weeds in sugarcane.

Keywords: Diuron, Herbicide, Hexazinone, Pre-emergent, Sugarcane, Weed Control

INTRODUCTION

Weeds contribute to substantial yield losses in sugarcane ranging from 6 % to 75%, and in some instances, up to total crop failure depending on the type of weed, degree, and duration of the competition (Witharama, 2000). As the early growth of sugarcane occurs at a fairly-slow rate, it takes about 3-4 months to develop a good canopy to cover the ground under irrigated conditions, and this period could be extended up to 4-5 months under rain-fed conditions in Sri Lanka. Thus, to raise a successful crop, weeds in sugarcane plantations have to be controlled until the crop develops a full canopy cover. Several options, such as manual, mechanical, cultural, and chemical methods, are available to control weeds in sugarcane. However, the adoption of integrated weed management is the best solution for controlling weeds in sugarcane (Bakker, 1990). Within integrated weed management systems, herbicides are essential (McMahon *et al.*, 2000), and different types of herbicides that are effective in controlling weeds under diverse field conditions must be made available for this. Mainly two types of herbicides, i.e., Pre-emergent and Post-emergent herbicides, are used for controlling weeds in sugarcane (Bakker, 1990). Pre-emergent herbicides are applied to the soil before weed and crop emergence, and post-emergent herbicides are applied 2-3 weeks after planting when the crop and the weeds emerge from the soil.

Diuron is a systemic herbicide easily taken up from soil solution by the root system of plants and rapidly translocates into stems and leaves by moving primarily via the xylem (Hess and Warren, 2002). Diuron inhibits the Hill reaction in photosynthesis, limiting the production of adenosine triphosphate (ATP) used for various metabolic processes. This process prevents CO_2 fixation, production of ATP, and other high-energy compounds that are needed for plant growth (Hess and Warren, 2002). Hexazinone inhibits photosystem II in the photosynthesis process of plants (WSSA-Herbicide Handbook, 1994). Hexazinone is absorbed from the soil solution by plant roots and translocated upward in the conductive tissues to the leaves, where it blocks photosynthesis within the chloroplasts (Ghassemi *et al.,* 1981). Most of the pre-emergent herbicides should be applied to soil when there is adequate soil moisture. Because free soil moisture is critical to the performance of most pre-emergent herbicides since pre-emergent herbicides that rely on root uptake will be less available in soil solutions with low soil water content (Congreve and Cameron, 2014).

Continued application of the same chemicals on the same site as herbicides could develop resistance of weeds to the herbicides, and application of herbicides with different modes of action will prevent the development of herbicide resistance (Mahmood *et al.,* 2014). Recently, the government of Sri Lanka banned the application of Glyphosate, a widely used effective herbicide in sugarcane fields (DOA, 2015). Hence, screening of newly developed herbicide formulations is essential to find out more effective herbicides for controlling weeds in sugarcane at different growth stages of the crop and the weeds. Therefore, the Sugarcane Research Institute (SRI) conducts studies to screen new herbicides to find out efficacious and economical herbicide treatments for weed control in sugarcane at different stages of crop and weed growth.

The experiments reported below were conducted to evaluate the effects of the new herbicide, Diuron 46.8 % + Hexazinone 13.2 % formulation (DI+HEX), for controlling weeds in sugarcane in terms of knock-down effect, residual activities, and crop safety. The optimal dosages of the herbicide and the appropriate time of application of herbicide for effective weed control in sugarcane were also investigated.

MATERIALS AND METHODS

The new herbicide

The new herbicide used for the study was a formulated product with Diuron and Hexazinone (DI+HEX). It contains 46.8 % Diuron and 13.2 % Hexazinone as active ingredients. The new product is a pre-emergent systemic herbicide, absorbed through roots and translocate within plants. It kills the plants by inhibiting the photosynthesis process and provides residual effects for controlling weeds.

Experimental procedure

Experiments to evaluate the herbicide were conducted in three different experiments during 2016 and 2017 at the Research Farm of SRI, Uda Walawe, and in sugarcane farmers' fields at Sevanagala. Initially, three herbicide treatments were chosen based on the dosages recommended by the manufacturer. Treatments were tested by spraying in observational plots at pre-emergent and early post-emergent stages of weeds. Based on the results of the observational experiment, three different doses of herbicide were subsequently tested in replicated experiments for detailed investigation on weed knock-down, residual activity, and crop damage by spraying at pre-emergent and early-post-emergent stages of weeds. The final experiment was conducted as a pilot project with three replicates in farmers' fields at Sevanagala to confirm the findings of the replicated experiments.

Description of the experimental sites

The experimental locations belonged to the Agro-Ecological Region, DL1a and DL1b (Punyawardane *et al.,* 2003). The soil in the area is predominantly well-drained Reddish Brown Earth (RBE) with a sandy clay-loam texture (Mapa *et al*., 2009). The area receives 1,300 mm average annual rainfall with 75 % expectancy, and about two-thirds of the annual rainfall is received from October to February (*Maha* season-second inter-monsoon and the Northeast monsoon). There is a small peak of rainfall from March to May (the first inter-monsoon and the start of the southwest monsoon). The ambient air and soil temperature are high and range from 28 °C to 32 °C. Considerably heavy weed growth with dominating grass and broadleaved weeds was observed in the experimental locations.

Observational experiment

Based on the manufacturer's recommendation, three different dosages of the new herbicide, DI+HEX, were selected to evaluate at pre-emergent and early-post emergent stages of weeds (Table 1). These three treatments were tested against the standard, Diuron 80 WP at the rate of 3.5 kg/ha and the non-weeded treatment in 100 m² plots. Spraying of herbicide was done after planting and before weeds or crop emergence at the pre-emergent stage. For the early-post-emergent stage, herbicide spraying was done at the 2-3 leaf growth stage of the weed and 10-12 days after sugarcane planting.

Table 1: Tested treatments at pre-emergent and early post-emergent stages in the preliminary experiment

Treatment No	Treatment Details	Dosage
1	DI+HEX	3.00 kg/ha
2	DI+HEX	3.25 kg/ha
3	DI+HEX	3.50 kg/ha
4	Diuron 80 WP	3.50 kg/ha
5	Control/Non weeded	-

Note: DI+HEX; Diuron 46.8 % + Hexazinone 13.2 % formulation

Replicated experiments

Two identical experiments, one for pre-emergent application just after planting sugarcane and the other for early post-emergent application at 12 days after planting (DAP) of sugarcane, were conducted in the 2016/2017 *Maha* season. Based on the observations made at the preliminary experiments, three dosages of the herbicide formulation were selected to test in the replicated experiments for detailed investigations (Table 2). Diuron 80% WP at the rate of 3.5kg/ha was applied as a standard treatment and un-weeded treatment was included as a control. The experiment was laid out in a randomized complete block design with three replicates, and the plot size was 9 m long, with six cane rows with 1.2 m row spacing. Spraying of herbicide was done after planting and before weeds or crop emergence at the pre-emergent stage. For the early post-emergent stage, herbicide spraying was done at the 2-3 leaf growth stage of weed and 10-12 DAP of sugarcane.

Table 2: Tested treatments at pre-emergent and early post-emergent herbicide application in the replicated experimen

	Pre-emergent application		Early post-emergent application	
Treatment No	Treatment Details	Dosage	Treatment Details	Dosage
1	DI+HEX	2.0 kg/ha	DI+HEX	2.5 kg/ha
2	DI+HEX	2.5 kg/ha	DI+HEX	3.0 kg/ha
3	DI+HEX	3.0 kg/ha	DI+HEX	3.5 kg/ha
4	Diuron 80 WP	3.5 kg/ha	Diuron 80 WP	3.5 kg/ha
5	Control/Non weeded	-	Control/Non weeded	-

Note: DI+HEX; Diuron 46.8 % + Hexazinone 13.2 % formulation

The experiment conducted in farmers' fields

Three farmers' fields in the Sevanagala rain-fed area, each with an extent of 2 ac, were selected for the study. Sugarcane was planted in the *Yala* season of 2017. Application of DI+HEX at the rate of 3 kg/ha at the pre-emergent stage of crop and weeds was identified as the most effective treatment based on previous experiments. Therefore, the above treatment was further tested on a pilot scale under farmer management conditions. Land preparation, planting, and crop management practices were carried out by the farmers. DI+HEX was applied at the rate of 3.0 kg/ha in half of the plot, and Diuron 80% WP was applied at the rate of 3.5 kg/ha in half of the plot as the standard treatment.

Establishment and maintenance of the experiments

Land preparation, planting, and crop management were carried out as per SRI recommendations (SRI, 1991). Seedbeds were prepared by making ridges and furrows with a tractor-mounted ridger with 1.2 m spacing between two rows. Three budded stem cuttings of variety Co 775 were planted in furrows and maintained under supplementary irrigation.

Herbicide application

Herbicides were applied by a hand-operated knapsack sprayer fitted with a single poly-jet nozzle. In the pre-emergent experiment, herbicides were sprayed on both ridges and furrows by a walking operator on the ridges. In the early-post emergent experiment, herbicides were sprayed only on ridges by a walking operator on the ridges. The swath width (45cm above the ground) was 1.5m. Spraying pressure was approximately 2-3 bars. The sprayer was calibrated before spraying, and the application rate was 400 L/ha. The herbicide treatments were applied when the soil was adequately moist.

Assessments

The effects of herbicide treatments on weed knock-down, residual activity and crop phytotoxicity were evaluated by visually and counting live weeds. The visual assessments made on weed control and crop damage were graded on a scale of 0 to 100 (Table 3). Live weeds present before and at regular intervals after introducing herbicide treatments were counted to estimate the effect of the treatments on weed knock down and residual activity. In the observational experiments, emerged weeds were counted in two months after spraying (MAS) of herbicide by placing a 40 x 40 cm quadrat in ten random places on the ridges of each treatment plot. In the case of the replicated experiment, the visual assessment was done at 2, 6, and 12 weeks after spraying (WAS), to find the effect of herbicide treatments on weed knock-down, residual activity, and crop phytotoxicity. The residual activity of the treatments was assessed in terms of their effect on weed control in comparison with the untreated control at 6 and 12 WAS. Each rating was the average of the minimum of three scores assigned by three different assessors. Also, weeds that appeared in each treatment plot at 2, 6, and 12 WAS were identified, counted, and recorded by placing 40 X 40 cm quadrat in a minimum of ten places selected randomly in each treatment plot. The number of sugarcane shoots that emerged in the inner four cane rows one month after planting (MAP) was recorded to measure germination. The number of tillers in four inner rows was counted at 2.5 and 3.5 MAP to estimate tiller production. The TVD (Top Visible Dewlap) height of 30 tillers selected randomly from the inner four cane rows in each treatment plot was recorded at 3.5 MAP to measure the tiller height. In the experiment conducted at farmers' fields, weed control was assessed both visually and by counting live weeds at 1, 2, and 3 months after spraying of herbicide treatments. The same procedures that were followed to give visual ratings and counting live weeds in the replicated experiments were followed in the farmer field experiments. Emerged weeds were counted in thirty random places for each treatment in each allotment in the experiment conducted in the farmers' field.

Table 3: Summary of the scale used for visual rating for weed control and crop damage

Scale	Degree of weed control	Degree of crop damage
0 – 10	No weed control	Minor crop damage
10 – 30	Poor weed control	Less crop damage
30 – 60	Moderate weed control	Significant crop damage
60 – 90	Satisfactory weed control	Severe crop damage
90 – 100	Complete weed control	Complete crop damage

Data analysis

The data were subjected to a normality test, and the data that followed the normal distribution were analysed with the ANOVA technique, and the data which were not followed the normal distribution were analysed with non-parametric techniques (Friedman's test).

RESULTS AND DISCUSSION

Observational experiments

Weed emergence was relatively low in all herbicide treatments applied for the pre-emergent study, and the effect increased with increasing the rate of application of DI+HEX (Table 4). Also, the effect of DI+HEX in controlling grasses and broadleaves was more than the sedges dominated by *Cyperus rotundus*. Moreover, the application of the new herbicide reported a high weed reduction percentage compared to the control and standard herbicide (Diuron 80 WP) application.

Table 4: The density of weeds at two months after pre-emergent application

Treatment details	Rate kg/ha	Weed Densities (Number/m^2)			Degree of weed control* (%)		
		G	S	BL	G	S	BL
DI+HEX	3.00	4	19	1	97	80	98
DI+HEX	3.25	2	14	1	98	85	98
DI+HEX	3.50	2	34	1	98	63	96
Diuron 80WP	3.50	11	6	6	91	94	81
Control		120	92	30	0	0	0

Note: DI+HEX; Diuron 46.8 % + Hexazinone 13.2 %, * Reduction of weed density compared to control, G: Grasses, S: Sedges, BL: Broadleaves

However, when DI+HEX was applied at the early post-emergent stage, weeds were effectively controlled to a satisfactory level for only up to one month (Table 5). Comparatively low weed control percentage was recorded even one month after application. Therefore, the effectiveness of DI+HEX in controlling weeds at the early-post emergent stage is low compared to pre-emergent application.

Table 5: The density of weeds at one month after early post-emergent application

Treatment details	Rate kg/ha	Weed Densities (Number/m^2)			Degree of weed control* (%)		
		G	S	BL	G	S	BL
DI+HEX	3.00	26	26	13	86	79	76
DI+HEX	3.25	22	23	14	88	82	72
DI+HEX	3.50	22	16	8	88	87	83
Diuron 80WP	3.50	27	9	15	86	93	71
Control		185	126	52	0	0	0

Note: DI+HEX; Diuron 46.8 % + Hexazinone 13.2 %, * Reduction of weed density compared to control, G: Grasses, S: Sedges, BL: Broadleaves

Replicated experiment

Pre-emergent application

As per the visual assessment, satisfactory weed control was observed until 6 WAS in all the treatments. However, at 12 WAS, weed control was low in Diuron 3.5 kg/ha treatment. The new herbicide formulation (DI+HEX) 3.0 kg/ha treatment recorded the highest weed control percentage both at 6 WAS and 12 WAS (Table 6).

Table 6: Visual ratings given for weed control at the pre-emergent stage

Treatment details	Rate kg/ha	Degree of weed control* (%)		
		2 WAS	6 WAS	12 WAS
DI+HEX	2.0	93 ±1.0	91±2.4	72 ± 2.6
DI+HEX	2.5	92 ± 1.2	94± 1.8	69 ±10.3
DI+HEX	3.0	98 ±0.7	99 ± 0.3	87 ±5.9
Diuron 80 WP	3.5	89±0.7	84± 2.0	54 ±6.3
Control	-	0	0	0

Note: DI+HEX; Diuron 46.8 % + Hexazinone 13.2 %, WAS; Week after spraying, * Reduction of weed density compared to control

Effect on weed densities

There is an increase in the emergence of weeds with time for all herbicide treatments, as indicated by estimated live weed densities at 2, 6, and 12 WAS. However, the weed emergence estimated at 2 WAS, was significantly ($p<0.05$) low at the pre-emergent application of 3.0 kg/ha of DI+HEX compared to the control (Table 7). The weed emergence in the plots sprayed with DI+HEX at the rate of 2.0 and 2.5 kg/ha and standard Diuron 3.5kg/ha were not statistically significant compared to the un-weeded control treatment. Further, as estimated at 6 WAS, the least weed emergence was observed in plots treated with DI+HEX at the rate of 3.0 kg/ha. Weed emergence for the new herbicide treatment 3.0 kg/ha was significantly less than the weed emergence in the plots treated with Diuron 3.5kg/ha and un-weeded control plots. However, at the 6 WAS stage, the application rates of 2.0, 2.5 and 3.0 kg/ha of DI+HEX were statistically similar (Table 7).

Table 7: Total weed densities in the field at two weeks, six weeks, and 12 weeks after application of treatments

Treatment	Rate kg/ha	Weed density (Number/m²)		
		2 WAS	6 WAS	12 WAS
DI+HEX	2.0	41 ^{ab}	68 ^{bc}	124 ^{ab}
DI+HEX	2.5	38 ^{ab}	54 ^{bc}	90 ^{bc}
DI+HEX	3.0	22 ^{b}	41 ^{c}	75 ^{c}
Diuron 80 WP	3.5	41 ^{ab}	87 ^{b}	133 ^{a}
Control		66 ^{a}	160 ^{a}	149 ^{a}
CV %		21.23	13.11	20.54

Note: *Means with the same letter in each column are not significantly different ($p>0.05$) WAS: Week after spraying, DI+HEX; Diuron 46.8 % + Hexazinone 13.2 %

Similarly, at 12 WAS, the least weed emergence was observed in plots treated with DI+HEX at the rate of 3.0 kg /ha. Estimated weed densities in the plots treated with DI+HEX at the rate of 2.5 and 3.0 kg/ha were not significantly different at 12 WAS. The effect of the new herbicide, 2.0 kg/ha dosage rate and standard Diuron 80% WP 3.5 kg/ha treatment were similar to the un-weeded control treatment at this stage (Table 7). Therefore, pre-emergent application of DI+HEX mixture at the rate of 3.0 kg/ha appears to be the most effective treatment of giving lasting residual weed control in sugarcane.

Effect on different weed species

Similar to the total weed densities, there is an increase in the emergence of different weed types at 2, 6 and 12 WAS for all herbicide treatments. But, the emergence of grasses and broadleaved species was low compared to the un-weeded control treatment (Table 8).

Table 8: Mean weed densities (Number/m^2) of grasses (G), sedges (S), and broadleaved weeds (B) at 2, 6 and 12 WAS

Treatment	Rate kg/ha	weed density (Number /m^2)								
		2 WAS			6 WAS			12 WAS		
		G	S	B	G	S	B	G	S	B
DI+HEX	2.0	0 ^{ab}	40	1 ^{b}	1 ^{b}	65 ^{b}	2 ^{b}	2 ^{b}	119	4 ^{b}
DI+HEX	2.5	0 ^{b}	37	1 ^{b}	0 ^{b}	53 ^{b}	1 ^{b}	2 ^{b}	84	3 ^{b}
DI+HEX	3.0	0 ^{b}	21	1 ^{b}	1 ^{b}	40 ^{b}	0 ^{b}	1 ^{b}	73	2 ^{b}
Diuron 80 WP	3.5	1 ^{ab}	36	4 ^{b}	3 ^{b}	80 ^{ab}	5 ^{b}	6 ^{b}	119	9 ^{b}
Control	-	5 ^{a}	32.	29 ^{a}	9 ^{a}	120 ^{a}	31^{a}	15 ^{a}	95	39 ^{a}
CV %		54.2	39.3	77.6	33.2	24.5	26.7	18.2	63.2	18.4

Note: *Means with the same letter in each column are not significantly different (p>0.05). DI+HEX; Diuron 46.8 % + Hexazinone 13.2 %

However, the emergence of sedges dominated by *Cyperus rotundus* was higher in all treatment plots. This indicates that neither standard Diuron treatment nor the tested rates of DI+HEX were effective enough to control *Cyperus rotundus* in the sugarcane field.

There is a reduction of densities of grasses, sedges, and broadleaved weeds compared to un-weeded treatment after application of all tested dosage rates of DI+HEX, and the effect is more than the standard Diuron 3.5 kg /ha (Table 9). Also, the reduction of densities of grasses, broadleaved weeds, and sedges has increased with increasing dosage rates of DI+HEX. However, the reduction of sedges density was less than the reduction of grasses and broadleaved weeds. In contrast, sedges density has increased in the plot treated with DI+HEX at the rate of 2.0 kg/ha and standard Diuron 3.5 kg/ha treatment. This may be due to the release of interference from emerged weeds since the pre-emergent application of the above two treatment have restricted the emergence of grasses and broad-leaved weed species but failed to suppress the emergence of *Cyperus rotundus*. Although DI+HEX was not effective in controlling sedges completely, it reduced the density of sedges significantly compared to the control at 6 WAS if applied at a high dosage rate.

Table 9: Reduction of weed densities at 6 and 12 weeks after application of treatments

Treatment	Rate kg./ha	Reduction in weed density* (%)					
		6 WAS			12 WAS		
		G	S	BL	G	S	BL
DI+HEX	2.0	91	46	94	87	-25	91
DI+HEX	2.5	98	56	97	85	11	92
DI+HEX	3.0	89	67	99	96	24	96
Diuron 80 WP	3.5	73	33	84	62	-25	77
Control	-	0	0	0	0	0	0

Note: G: Grass, S: Sedge, BL: Broadleaves and the minus value indicates that increase of weed density, DI+HEX; Diuron 46.8 % + Hexazinone 13.2 %, WAS: Week after spraying, * Reduction of weed density compared to control

Effect of the herbicide on sugarcane growth

There were no phytotoxicity symptoms observed visually in the experiment sprayed with pre-emergent treatments. The average number of emerged shoots at 1 MAP was 6 per meter row length, and there were no significant differences (p > 0.05) in the values between different treatments. Also, the tiller production at 2.5 and 3.5 months after planting was not significantly different between plots applied with herbicide treatments. However, tiller production in the un-weeded control treatment was significantly (p<0.05) low than the other treatments. (Table: 10) This may be due to suppression of tiller production due to the interference of emerged weeds in un-weeded control plots. Also, there was no significant difference in plant TVD height recorded at 3.5 MAP between different herbicide treatments. But significantly higher plant height was recorded in herbicide-applied treatments compared to the control. This confirmed that sugarcane growth was not affected due to the application of tested rates of DI+HEX herbicide.

Table 10: Tiller production and plant height at different rates of application of herbicide and time after planting of sugarcane

Treatment	Rate kg/ha	No of tillers/m		Plant TVD height (cm)
		2.5 MAP	3.5 MAP	3.5 MAP
DI+HEX	2.0	11 [a]	10 [a]	97 [a]
DI+HEX	2.5	11 [a]	11 [a]	96 [a]
DI+HEX	3.0	12 [a]	13 [a]	99 [a]
Diuron 80 WP	3.5	11 [a]	10 [a]	98 [ab]
Control	-	6 [b]	6 [b]	81 [b]
CV %	-	14.7	11.4	8.2

Note: *Means with the same letter in each column are not significantly different ($p>0.05$). DI+HEX; Diuron 46.8 % + Hexazinone 13.2 %, MAP: Month after planting,

Early-post emergent experiment

Visual observations

According to the averages of the ratings given for weed control at 2 WAS, the highest value (73%) was recorded for DI+HEX applied at the rate of 3.5 kg/ha. But the effect on weed control has reduced with time, as depicted by the ratings (58%) given at 2 MAS. Therefore, weed control at 2 MAS of DI+HEX was not satisfied (Table 11). However, the phytotoxicity showed by DI+HEX was minor and negligible.

Table 11: Visual ranking for weed controlling in the early-post emergent experiment

Treatment details	Rate kg./ha	Degree of weed control* (%)	
		2 WAS	2 MAS
DI+HEX	2.5	73 ± 7.0	53 ± 1.7
DI+HEX	3.0	70 ± 7.7	60 ± 4.4
DI+HEX	3.5	73 ± 5.1	58 ± 2.0
Diuron 80 WP	3.5	62 ± 1.0	51 ± 2.5
Control	-	0	0

Note: DI+HEX; Diuron 46.8 % + Hexazinone 13.2 %, WAS: Week after Spraying, * Reduction of weed density compared to control

Effect on weed densities

Weed densities of different weed species have not been reduced to a satisfactory level even one month after the application of DI+HEX (Table 12). In contrast, the total weed densities of each treatment have increased with time. Also, at 2MAS, all treatments were statistically similar to the control treatment. This confirmed that the application of DI+HEX at the post-emergent stage is not effective in controlling weeds in sugarcane fields.

Table: 12 Total weed density at different times after spraying for early-post emergent study

Treatment	Rate kg/ha	Weed Density (Number/m^2)		
		Before application	1MAS	2 MAS
DI+HEX	2.5	42	48 [b]	49
DI+HEX	3.0	73	54 [b]	60
DI+HEX	3.5	57	105 [a]	66
Diuron 80 WP	3.5	41	48 [b]	54
Control	-	78	121 [a]	69
CV		ns	62.3	ns

Note: DI+HEX; Diuron 46.8 % + Hexazinone 13.2 %, MAS: Month after Spraying

The experiment conducted in farmer's field at Sevanagala

According to the visual observations made at 1 and 2 MAS, more weed control was observed in DI+HEX applied area compared with the standard Diuron 80 % WP 3.5 kg/ha (Table. 13). Also, the residual effect of DI+HEX at the rate of 3.0 kg/ha was high. This is because the herbicide treatment has given satisfactory weed control over 2 MAS, and the degree of weed control is more than the standard Diuron 3.5 kg/ha treatment.

Table 13: Visual ranking for weed control at farmer's fields at Sevanagala

Treatment details	Rate kg./ha	Degree of weed control (%)	
		1 MAS	2 MAS
DI+HEX	3.0	90.5 ± 2.1	88.25 ± 1.2
Diuron 80 % WP	3.5	80.75 ± 1.5	64.25 ± 2.2

Note: DI+HEX; Diuron 46.8 % + Hexazinone 13.2 %, MAS: Month after Spraying

There were no significant differences in emerged weed densities between the two treatments at 1 MAS. But, the plots treated with DI+HEX at the rate of 3.0 kg/ha have recorded significantly low weed densities at 2 and 3 MAS (Table 14). Therefore, the residual activity of DI+HEX is significantly higher compared to Diuron 80 WP.

Table 14: Total weed density at 1 MAS, 2 MAS, and 3 MAS in farmer's field at Sevanagala

Treatment details	Rate kg/ha	Weed Density (Number/m^2)		
		1 MAS	2 MAS	3 MAS
DI+HEX	3.0	6	12 ^{b}	19 ^{b}
Diuron 80 % WP	3.5	7	21 ^{a}	30 ^{a}

Note: *Means with the same letter in each column are not significantly different (p>0.05) DI+HEX; Diuron 46.8 % + Hexazinone 13.2 %, MAS: Month after spraying

CONCLUSION

The results of the experiments confirmed that the application of a new herbicide formulation, Diuron 46.8 % + Hexazinone 13.2 % (DI+HEX) at the pre-emergent stage, was effective in controlling grass and broad-leaved weeds in sugarcane fields and the efficacy of this herbicide treatment is better than the standard Diuron 80 WP 3.5 kg/ha treatment. However, this formulation is not effective in controlling sedges, particularly *Cyperus rotundus*. Therefore, the application of DI+HEX formulation at the rate of 3.0kg/ha mixed with 400 L water at the pre-emergent stage is recommended to control grass and broadleaved weeds in sugarcane plantations.

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Rainfall Pattern Changes in Sugarcane Plantation in Sevanagala, Sri Lanka

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ABSTRACT

The present study was carried out to identify temporal changes in rainfall patterns in the Sevanagala sugarcane project area in Sri Lanka. Rainfall data collected from the agro-meteorological station of the Sevanagala sugarcane project from 1984 to 2018 were taken for analysis. Dates of rainfall onset and date of terminations, and length of rainfall seasons were assessed. The single mass curve method was used to confirm the consistency of the data set. The Mann-Kandal test was conducted to identify of statistical significance of changes in rainfall onset date, date of termination, and length of rainfall season. The time series trend of the rainfall onset date, date of termination, and length of rainfall season were quantified using Sen's slope estimation method. The results revealed that bi-modal rainfall distribution is prominent in the Sevanagala sugarcane project area. On average, the first rainfall season is distributed from March to July, and the second rainfall season is from October to February. Analogically, the rainfall season onset date for the first and second rainfall seasons was the twenty-eighth of March and the fourteenth of October, respectively. Termination dates of the first and second rainfall seasons were the twenty-ninth of May and the twenty-ninth of December, respectively. Based on the Mann-Kandal test, it was revealed that the rainfall season onset date in the first rainfall season was significantly delayed at a rate of 2.6 days per decade during the 1984 to 2018 period. The termination date of the first rainfall season did not exhibit any significant change over time during the study period. The rainfall onset date of the second rainfall season was also shown a significant delaying trend by 2.7 days per decade during the respective 35-year period. The length of the first rainfall season has not shown a significant change over time. The length of the second rainfall season has shown a significant change by shortening its length at a rate of 7.6 days per decade. Hydro-climatological challenges incurred due to these changes in rainfall season onset date and season length can have a negative effect on the soil water balance of the sugarcane plantations. Thus, precautions must be taken to mitigate this issue at the farmer's field scale as well as the water-basing scale. Most adverse hydro-climatological conditions can often be eliminated by completing land preparation activities in advance and planting operations immediately after starting of rainfall.

Key word: Climate change, Rainfall pattern, Sri Lanka, Sugarcane

INTRODUCTION

Changing rainfall pattern has been identified as a major climatic issue in crop production in Sri Lanka (Esham and Garforth, 2012; Panabokke and Punyawardena, 2010). This effect is more significant in crop cultivations that follows rainfall pattern for their planting and harvesting operations (Zhao and Li, 2016). Rain-fed sugarcane, which has an annual crop cycle, is one such crop cultivated in the dry zone of Sri Lanka (Shanmuganathan, 1990). Most field operations, including land preparation, planting, and other agronomic practices, are seasonal practices in rain-fed sugarcane cultivations (Kumarasingheand Wijayawardhana, 2011). Two planting seasons, from March to April (first planting season) and from October to November (second planting season), and the short harvesting season from January to March and the main harvesting season from May to October, are usually practiced (Shanmuganathan, 1990).

Sugarcane planting at inappropriate times due to erratic or delayed rainfall seasons has caused germination failures, uncontrollable weed growth, and suppression of tillering (Wyseure *et al.*, 1994). Also, late planting directly causes great economic losses at seed cane nurseries as over-matured nurseries often have to be harvested for crushing cane. Rainfall established in advance is another negative impact of rainfall variations, as it interrupts the land preparation activities and increases the risk of uncontrollable soil erosions in newly tilled sugarcane lands. Not only the onset date, date of termination, and length of rainfall period are also to be considered as delaying of date of termination of rainfall season directly influences the starting date of harvesting season of sugarcane plantations. At least one month of dry weather conditions is needed for the accumulation of sugar in sugarcane stalks (Mettananda, 1990; Wyseure et al., 1994). Nevertheless, dry weather is needed for safer transport of sugarcane harvest (Shanmuganathan, 1990), as most of the furrow lines within the sugarcane field are subjected to destruction during transport operations of cane harvest under moist weather conditions. Thus, planning the planting and harvesting schedules based on scientific judgments on rainfall season onset date, date of termination, length of the rainfall season, and their changes over time is a fundamental need to ensure the sustainable management of sugarcane plantations in Sri Lanka. Therefore, the current analysis was carried out to find out the onset date, date of termination, and length of rainfall season and quantify their changes in the Sevanagala sugarcane plantation site based on the daily rainfall data over the past three decades.

MATERIALS AND METHOD

Study area

The study was conducted in the Sevanagala sugarcane project in Sri Lanka (latitude from 6°26'36.53"N to 6°20'4.35"N and longitude from 80°50'31.94"E to 80°58'10.23"E). The area locates on the western boundary of Moneragala district, 150 km away from the capital Colombo. It is located in the low country dry zone (DL_{1b}) as per the agroecological zone classification (Punyawardhana, 2008). Usually, two rainfall seasons per year are prominent. The mean annual temperature is about 28.6 ^{0}C, and the average humidity is 72.6%. Average pan evaporation is about 4.5 mm/day (Wijayawardhana *et al*., 2013).

Data

Daily rainfall data recorded in the agro-met station of the Sevanagala sugarcane project, Sri Lanka (Latitude: 6°23'46.93"N; Longitude: 80°54'45.54"E) for 35 years from 1984 to 2018 were used for the analysis. Two rainfall seasons (in local name, *YALA* season and *MAHA* season rainfall) were analyzed separately. Day number was given from 1 to 184 for the first rainfall season, starting from the 1^{st} of March till the 31^{st} of August, and from 1 to 182 for the second rainfall season, starting from the first of September to the 28^{th} or 29^{th} of February, respectively. The consistency of the data set was tested with the single mass curve method(Kazembe, 2014).

The long-term average rainfall

The long-term average was evaluated based on the mean monthly and 75 probability rainfall method. 75 probability rainfall was calculated as per the ranking method (Ritzema, 1994) using equation 01.

$F(x>x_r) = 1-[r/(n+1)]$--------------.Equation 01

Where, $F(x \geq x_r)$ = 0.75, r = rank number, n = number of years in the dataset

Rainfall seasons onset and date of termination

Several methods have been used to define rainfall onset date of a rainy period (Matthew *et al*., 2017; Odekunle, 2006), *i.e*., point of the maximum curvature of cumulative rainfall curves (Sonnadara, 2015) or point at the 7-8 % cumulative percentage (Amekudzi *et al*., 2015; Matthew *et al*., 2017; Odekunle, 2004) or probability or dependable rainfall method (Weerasinghe, 1989) or water balance method (Kazembe, 2014). In the current paper, the date at the 10 % of cumulative rainfall was taken as the rainfall onset date of the season, and similarly, the date at 90% of accumulated rainfall was selected as the date of termination. Rainfall onset and date of termination in the first rainfall season in 1995 are depicted in Figure 1 to demonstrate the methodology followed in selecting the onset and date of termination.

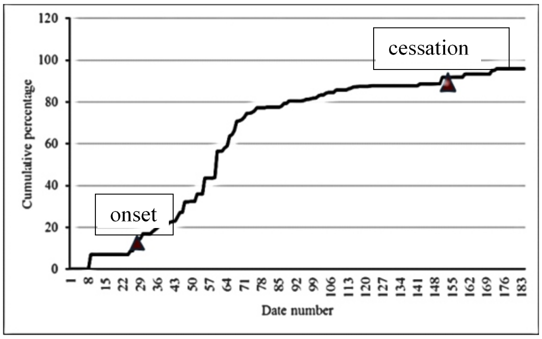


Figure 1: Rainfall onset date (27= 27th march) and date of termination (151 = 29th July) in first rainfall season, 1995

Rainfall seasons length

The length of the rainfall season was obtained using the following equation.

L= (dt-do)+1 --------Equation 02

Where, L = Rainfall season length (number of days), dt = date of termination, do = onset date

Statistical analysis

Statistical analysis was carried out with MAKESENS software developed by Finnish Meteorological Institute (2002). The significance of increasing or decreasing trend for the rainfall season's onset date, date of termination, and rainfall season length was tested with the Man-Kandal procedure (Ojo and Ilunga, 2018) and was evaluated using Z distribution at alpha values of 0.05, 0.1, and 0.2. A positive Z value indicates an upward trend, and similarly negative values for downward (Salmi *et al*., 2002). The adopted model is given in Equation 3.

$x_i = f(t_i) + \varepsilon_i$ ------- Equation 03 (Salmi *et al*., 2002)

Where f(t) = increasing or decreasing function of parameter x (x= onset date or date of termination or length of the rainfall season), ε_i = residual component.

The slope of the linear trend (as change per year) was estimated with the Sen's method (Ojo and Ilunga, 2018). In Sen's method, the slope of all data pairs of the time series data set is calculated individually (Equation 04), and then the parameter Qi is ranked according to the ascending order. The median value of the ranked data set was taken as the final value of the slope of the trend line (Salmi *et al*., 2002).

Where, Q_i= slope between desired data pair, (x_j- x_k) =desired data pair, $(j-k)$ = interval between x_j and x_k (number of days)

$$Q_i = \frac{(x_j - x_k)}{(j-k)}$$ --------Equation 04

RESULTS

The long-term average rainfall

The distribution of mean monthly rainfall and 75 probability rainfall is given in the Figure 2. According to the mean monthly and 75 probability rainfall data, a bi-

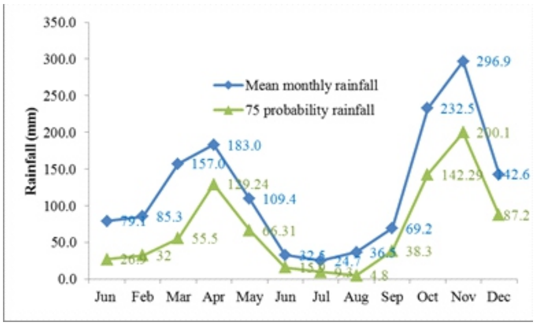


Figure 2**:** Distribution of mean monthly rainfall and 75 probability rainfall in Sevanagala (data 1984-2018)

modal rainfall pattern with two peaks in April and November was prominent in the Sevanagala sugarcane plantation site.

The mean monthly rainfall of April and November are 183.0 mm ± 14.4 mm and 296.9 mm ± 20.7 mm, respectively. July

was the driest month, having the lowest mean monthly rainfall of 24.7 mm ± 4.1 mm. Annual average rainfall from 1984 to 2018 was 1448.6 mm ± 49.4 mm.

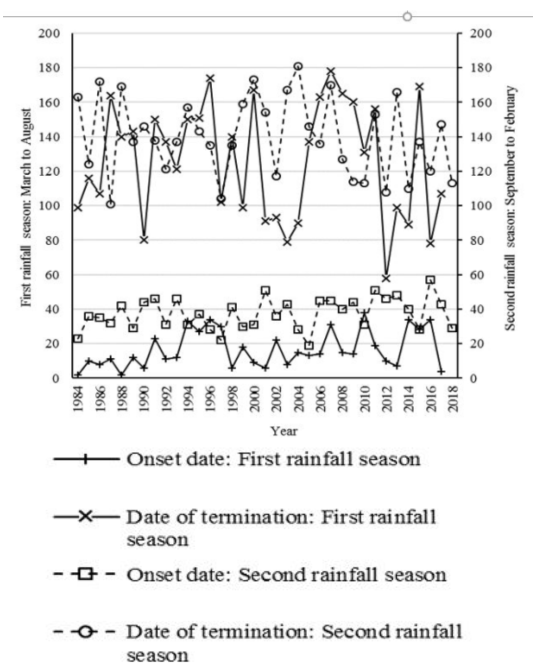


Figure 3: Distribution of onset date and date of termination of two rainfall seasons (data from 1984-2018)

Onset date, date of termination, and length of rainfall seasons

The distribution of onset date, date of termination, and length of rainfall seasons from 1984 to 2018 are given in Figure 3.

First rainfall season

The first rainfall season starts by the twenty-eigh[th] of March and continues till the twenty-nin[th] of May at a probability ≥ 0.75 level. As such, there is a 25% chance to extend the first rainfall season after the thirtieth of May. These findings are highly compatible with the previous studies (Ariyawansha and Keerthipala, 2010).

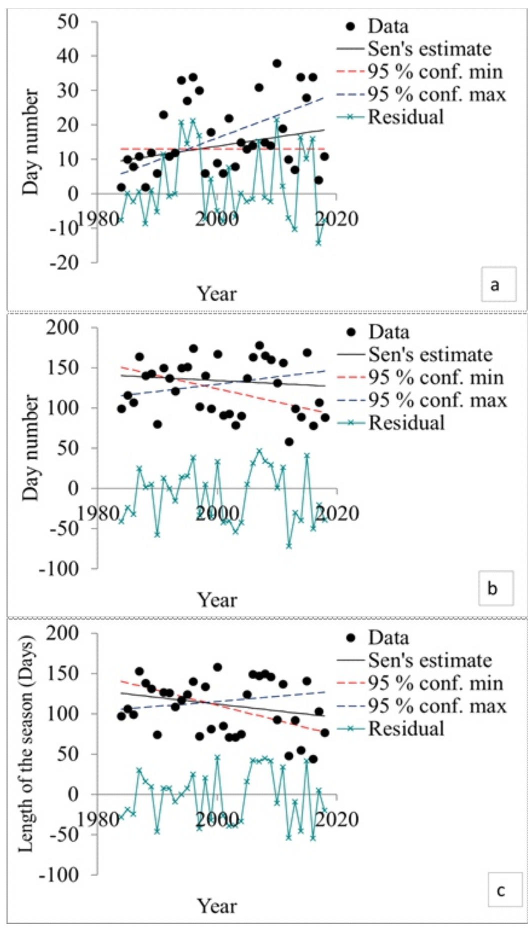


Figure 4: Temporal changers of onset date (a), date of termination (b) and length (c) of the first rainfall season

However, on average, the first rainfall season receives 543.0 mm ± 22.1 mm rainfall which constitutes 37.5 % of the annual average rainfall. Temporal variations of the onset date, date of termination, and length of rainfall seasons (duration) of the first rainfall season are shown in Figure 4.

Constructed trend lines (Sen's estimate) confirm that the onset date of the first rainfall season is an in-delaying trend (significant at 0.1 alpha level). Parameters for the trend lines of the first rainfall season are given in Table 2.

Table 2: Trend lines statistics for first rainfall season

Parameter	Calculated Z value	Regression line gradient (Sen's slope estimate)	Relative change per decade (days)
Onset date	1.71**	0.263	2.63
ceeasation date	-0.70	-0.056	0.56
Rainfall season length	-0.98	-0.094	0.94

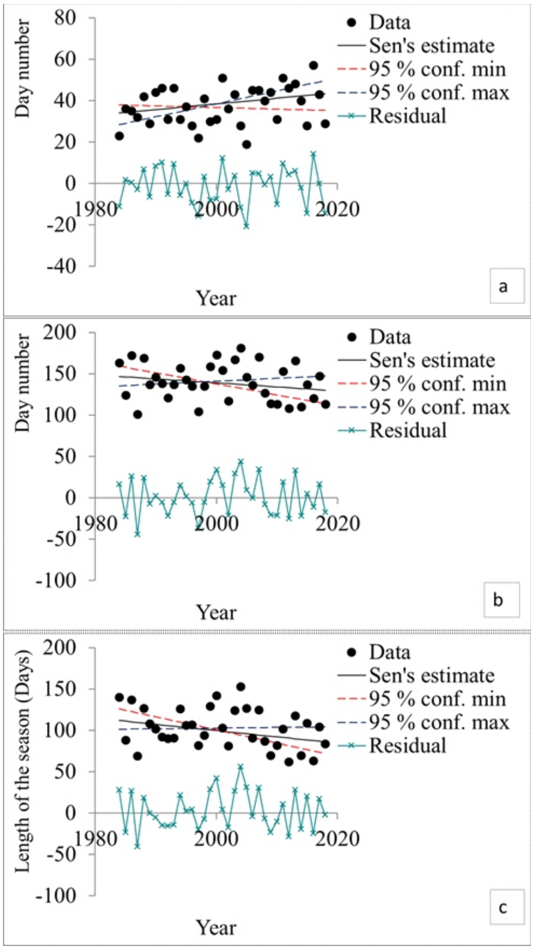
****Significant at α = 0.05, ** Significant at α = 0.1, * Significant at α = 0.2*

It is evident that (Table 2) the onset date of the first rainfall season has been delayed at a rate of 2.63 days per decade. However, the date of termination and length of the first rainfall season has not exhibited a significant change during the study period due to a high standard error at ± 6 on the temporal scale.

Second rainfall season

According to the analysis, the second rainfall season starts on the fourteenth of October and continues till the twenty-ninth of December (probability ≥ 0.75). On average, 905.7 mm ± 36.0 mm of rainfall is received during the second rainfall season, and it constituted 66.7% which is higher than that of the rainfall in the first rainfall season. The second rainfall season receives 62.5% of the annual average rainfall. Temporal changes of onset, date of termination, and length of season (duration) of the second rainfall season are shown in Figure 5.

It is evident that the onset date of the second rainfall season is in a delaying



Figuer 5: Temporal changers of onset date (a), date of termination (b) and length (c) of the second rainfall season

was observed to be decreasing trend at alpha=1.0 significant level. Parameters for the trend lines of the second rainfall season are given in Table 3.

Table 3: Trend lines statistics for the second rainfall season

Parameter	Calculated Z value	Regression line gradient (Sen's splope estimate)	Relative change per decade (days)
Onset date	1.41*	0.273	2.73
Ceeasation date	-1.19	-0.485	4.85
Rainfall season length	-1.72**	-0.759	7.59

****Significant at α = 0.05, ** Significant at α = 0.1, * Significant at α = 0.2*

Accordingly, the onset date of the second rainfall season has been significantly delayed at a rate of 2.73 days per decade. Also, it was worth noting that the length of the rainfall season has diminishing trend at a rate of 7.59 days per decade for the period of 1984 to 2018. However, the trend of the date of termination of the second rainfall season was not significant.

DISCUSSION

The narrowing of the rainy season due to delayed starting and the early termination of the rainy season can adversely affect the sugarcane replanting calendar currently being implemented in Sevanagala. It has also been observed that the shortening of the rainy season leads to an increase in the length of the dry period. This phenomenon can be identified as an emerging threat to young cane plants. In addition, shortening the duration of the rainy season puts additional pressure on subsequent field operations, such as land preparation and timely supplying of plant material from the nurseries, as most of these operations may be completed during the shortened rainy season. Moreover, operations linked to water management and soil conservation can be hampered due to the intensification of field operations, which ultimately leads to serious damage to soil resources in long term.

Therefore, short-term and long-term strategies must be adapted to address the above-mentioned hydro-climatological challenges associated with changes in rainfall onset dates and length of the rain season. Completing land preparation activities in advance and planting operations immediately after the commencement of the rainy season are possible strategies that can easily be adopted at the farmer's fields scale to meet these hydro-climatological issues. Providing adequate machinery facilities, including high-powered tractors for land preparation during the dry season, may be an additional solution to improve land preparation before the onset of rainfall because it allows to take full advantage of the rainfall in terms of germination tillering and growth of the sugarcane. It enables to utilize complete rainfall season for crop establishment and growth.

CONCLUSION

The daily rainfall data analysis of the Sevanagala sugarcane plantation site for 1984–2018 demonstrated a characteristic bimodal rainfall pattern. Dates of the onset of the rainfall season are observed to be delayed by 2.6 and 2.7 days per decade during the first and second rainfall seasons, respectively. However, the date of termination of both rainfall seasons remained the same.

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Ovipositional Preference of *Deltocephalus menoni* (Hemiptera: Cicadalidae), Vector of Sugarcane White Leaf Disease in Sri Lanka

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ABSTRACT

Information on the biology and ecology of *Deltocephalus menoni* (Homoptera: Cicadelidae), the only vector identified as responsible for spreading the White Leaf Disease (WLD)-causing phytoplasma in sugarcane in Sri Lanka, is essential to develop an effective disease management programme. Laboratory experiments involving choice tests and field studies were conducted at the Sugarcane Research Institute (SRI), Uda Walawe and sugarcane fields in Pelwatte from 2014 to 2017 to find out the ovipositional preference of the vector on six types of soil textures, with filter-mud and/or spent wash incorporation, with polythene mulches and plants mulched with sugarcane trash. The data on the number of eggs in different substrates were collected 14 days after the introduction of the vector. The differences in the number of eggs in different substrates were analyzed by the analysis of variance procedure and Duncan's multiple range test. The results revealed that the vector preferred sandy loam, fine sand soil, and filter-mud-incorporated soil for laying eggs. Mulching sugarcane trash did not show any significant effect on oviposition. Black and transparent polythene manage the oviposition significantly at the laboratory level but no significant effect of black polythene mulch was recorded under field conditions. Significantly higher rate of oviposition compared to the control recorded in filter-mud and spent wash incorporated fields into the soil. The findings confirmed that more attention should be given to sugarcane crops in the fields with sandy-loam and fine-sand soils in managing the disease. The incorporation of filter mud and spent wash into the soil should be minimised in the fields where WLD incidence is high. Rogueing out of the WLD-infected plants is essential to make the habitat unfavorable for laying eggs by the vector.

Keywords: *Deltocephalus menoni*, Ovipositional preference, Sri Lanka, Sugarcane White

INTRODUCTION

Until recently, the plant diseases caused by phytoplasma diseases have been managed by spraying insecticides, with or without managing the vector(s). Vector management by habitat manipulation has been identified as one of the effective strategies of the new integrated approach for managing phytoplasma diseases (Weintraub and Wilson, 2010). *Deltocephalus menoni* (Hemiptera: Cicadellidae, Subfamily: Deltocephalinae) is the only vector identified so far transmitting the sugarcane White Leaf Disease (WLD) in Sri Lanka (Senevirathne *et al.*, 2008). Therefore, management of the vector *D. menoni* is a prerequisite to managing this devastating disease (Kumarasinghe and Jones 2001; Senevirathne *et al.,* 2006; Chanchala *et al.,* 2014; Chanchala *et al.,* 2015).

Vector-borne diseases of several crops have been managed successfully by disrupting the lifecycle of the vectors by manipulating their egg-laying habitats (Howard and Oropeza, 1988; Summers and Stapleton, 2002; Mannini, 2007). An integrated approach developed to control the X-disease of stone fruits suggests that the management of ground cover in the orchards is an effective cultural measure for reducing leafhopper vector populations and feeding damage to peaches (Douglas and McClure, 1988). It has been identified that the insect vector of grapevine yellows lays eggs under the barks of grapevine. After identification, both phytoplasma and the eggs of the vector were simply eliminated by treatment with heat (Cauudwell, 1966). The current practice is treating grape vines with hot water (Mannini, 2007). The insect vector of bois noir disease of grapevine *Hyalesthes obsoletus* oviposit at or just below the soil surface, and vector movement into the soil has been physically prevented by using synthetic mulches such as plastic sheeting (Maixner, 2007). Summer and Stapleton (2002) reported that better control of maize leaf hopper *Dulbulus maidis*, a vector of maize stunt, leads to a higher maize yield. The control has been achieved by using plastic reflective mulch in which oviposition in the soil had been prevented. Similarly, the aster yellow phytoplasma vector on carrot, *Macrosteles quadrilineatus,* has been controlled by laying aluminum foil mulch to prevent oviposition by the vector (Stewan and Ragsdale, 1987).

D. menoni oviposits in the soil near the sugarcane plants, occasionally on the leaf sheaths at the base of the plants and on sugarcane trash (Senevirathne *et al.,* 2008). Information on its preference for laying eggs in different substrates has not been documented. Therefore, this study was conducted with the objective of determining the ovipositional preference of *D. menoni* in the different soil textures, amendments and mulches for formulating the management strategies of WLD in sugarcane in Sri Lanka.

MATERIALS AND METHODS

Laboratory and field studies were conducted to determine the ovipositional preference of the *Deltocephalus menoni* with different soil textures, amendments, and mulches.

I. six different soil textures (gravel, coarse sand, fine sand, sandy loam, clay loam and clay)

II. soils incorporated with filter mud and spent wash

III. soil mulched with sugarcane trash and

IV. soil mulched with polythene

Four laboratory experiments were conducted in insect-proof rearing cages in the entomology laboratory of the Sugarcane Research Institute (SRI), Uda Walawe, keeping the inside temperature at 25-30^{0}C, relative humidity at 70 - 80% and under natural light to determine the ovipositional preference of *D. menoni.*

Field experiments were conducted in farmer fields in Sevanagala, Ginigalpelessa area (i), Pelwatte, Menik-Ganga nursery area (ii) and research farm (iii & iv) of the Sugarcane Research Institute (SRI), Uda Walawe.

The experiments on ovipositional preference of *D. menoni* in different soil textures were repeated three times covering both cropping seasons *Maha* 2014/15 (Mid-September to Mid-March) and *Yala* 2015 (Mid-March to Mid-September). The other three experiments on the ovipositional preference of *D. menoni* in filter mud and spent wash as soil amendments, sugarcane trash and polythene as soil mulch and infected sugarcane plants were conducted three times from February to April 2015. Field experiments were conducted from 2015 to 2017.

The following procedures were followed throughout the research period to maintain insect cultures and test plants

Maintaining insect cultures

The adult insects of *D. menoni* were collected, using a sweep net and a pooter, in sugarcane fields of less than six months old in the research farm, SRI, Uda Walawe. The insects collected were reared in insect-rearing cages according to the protocol developed by Senevirathne *et al.,* 2008).

Maintaining healthy plants

Healthy seed cane of the variety SL 96 128 was obtained from a well-maintained nursery raised using hot water-treated (dipping seed cane in 54^{0}C hot water for 50 minutes) seed cane, by considering the visual symptoms of the plants (Chandrasena *et al.,* 2003; Senevirathne, 2008). Single-budded setts were again treated with hot water for further assurance of free of the phytoplasma, and the plants were maintained in insect-proof field cages under the recommended agronomic practices (SRI, 2004).

Preparation of experimental pots

Healthy and WLD symptomatic plants grown in plastic pots (14 x14cm) were separately maintained in insect-proof cages until three months of age. The plants were thoroughly cleaned by removing ants, spiders, and other insect predators before introducing treatments. The soil surface of each pot was covered with polythene as underpinnings to pave egg-laying substrates for the experiment.

I. Ovipositional preference of *D.menoni* in different soil types

a. Laboratory study

Six substrates representing different soil textures, viz., gravel, coarse sand, fine sand, sandy loam, clay loam and clay were used for the experiment. Sandy loam and clay-loam soils were selected based on laboratory analysis. The river sand sieved by 0.35 mm sieve was used as fine sand and the portion left above 0.35 mm sieve and passed through 0.59 sieve was used as coarse sand. Gravel and clay substrates were selected by looking at the physical appearance and nature of roughness while touching soil samples. All substrate samples were sterilised to eliminate other entomophagous and entomopathogenic soil fauna and were paved on the polythene sheet to make a 2cm thick layer below each plant. Six pots with a single plant in each, and each containing one testing substrate were enclosed in insect-proof rearing cages (50 x30 x 20cm) and were arranged randomly in a cage for multiple-choice tests. Ten gravid females of *D. menoni* were introduced to the cage and maintained for two weeks for oviposition. After 2 weeks in the insect-proof rearing cages, the substrate in each pot was collected separately and examined through a light microscope (KYOWA TOKYO, 10x3). The number of eggs found in each substrate was counted and recorded. The experiment procedure was repeated six times; three times in the *Maha* 2014/15 season and three times in the *Yala* season 2015.

b. Field study

A field survey was conducted in farmer fields in Sevanagala (Ginigalpelessa area). Thirteen (13) farmer fields with plant crop of variety SL 96 128 was used for the study and which was located among WLD-infected sugarcane fields where vectors were available naturally. 25x10m^{2} size 6 plant plots were considered for the study. A random sampling technique was used to collect soil samples from 0-15 cm depth by using a soil augur. Five soil samples were collected from each plot and one composite sample was prepared for final analysis representing each farmer field. Collected samples from 13 farmer fields were analyzed for their textural classes followed by the hydrometer method. Vector populations in each plot were recorded in monthly intervals from 3 to 5 months of age as, the number of vectors captured for 500 sweeps within the plot during ratoon I and ratoon II crops.

II. Ovipositional preference of *D. menoni* in soils incorporated with filter-mud and spent wash

a. Laboratory study

Filter-mud

Fresh filter mud, 2 – 3 days after disposal from the factory, was used as the substrate. Ten pots with healthy plants were selected for the experiment. A 2cm thick, dried filter-mud layer was paved on the polythene sheet placed on the soil surfaces as the egg-laying substrate in five pots. The remaining five pots were used to pave with a 2cm thick, sterilised sandy loam soil layer (Steamed at 100°C for 3hrs) as the control. Both the filter-mud and soil-paved pots were enclosed in an insect-proof rearing cage for a choice test. The newly-emerged five adult vectors (3 females: 2 males) were introduced to each cage and left for two weeks for oviposition. The oviposition was evaluated as explained in experiment 1. Treatments were replicated fifteen times.

Spent wash

Healthy sugarcane plants grown in plastic pots (14 x14cm) were maintained in insect-proof cages until three months of age. The plants were thoroughly cleaned before introducing treatments. The soil surface of each pot was covered with polythene as underpinnings to pave egg-laying substrates for the experiment. Spent wash obtained from the Sevanagala sugar mill was used for the experiment, and 5 ml of spent wash was mixed thoroughly with the same amount of sandy loam soil paved on each plant plot to make spent wash incorporated soil substrate. Ten pots (single plant in each) were used to pave with a 2cm thick layer of soil and similarly, ten plots with a single plant in each were used to pave each with a 2cm thick layer of sterilised sandy-loam soil as the control. One pot with spent wash incorporated soil and one pot with normal soil-paved pots were enclosed together in an insect-proof rearing cage for the choice test. The newly-emerged five adult vectors (3 females: 2 males) were introduced to each cage and left for two week for oviposition. After 2 weeks insects were removed from cages and the substrate in each pot was collected separately. Soil samples were separately examined through a light microscope (KYOWA TOKYO, 10x3). The number of eggs found in each substrate was counted and recorded.

b. Field study

The study was conducted in the Menik-Ganga nursery in Lanka Sugar Pelwatta Pvt (Ltd). Sugarcane fields at 4 month age and variety SL 96 128 were selected with the following treatments *ie.* filter mud incorporated fields (20 tons/ha), spent wash incorporated fields (40 L^3), filter mud (20 tons/ha), and spent wash incorporated fields (40 L^3) and fields without any incorporations. Three plots were considered for each treatment. Vector populations in each plot were recorded in weekly intervals during the four-month age as, the number of vectors captured for 500 sweeps within the plot during the plant crop stage.

III. Ovipositional preference of *D. menoni* in soil mulched with sugarcane trash

a. Laboratory study

Sterilised soil mulched with trash and without trash mulch as control was used as the oviposition substrates. The sugarcane trash composited with dried sugarcane leaves and leaf sheaths collected from a newly-ratooned field of the variety SL 96 128 was used for the study. A 2cm height layer of the substrates was paved on a polythene sheet laid on each pot, A pair of pots with mulched soil and un-mulched soil was kept enclosed in an insect-proof rearing cage (45 x 30 x 30cm). The newly-emerged five adult vectors (3 females: 2 males) were introduced to each cage and left for two weeks for oviposition. The same procedure was repeated fifteen times. The oviposition was evaluated as explained in experiment 1.

b. Field study

The field survey was conducted in Research Farm (SRI), Uda Walawe. A ratoon crop of variety SL 96 128 was used for the study and was located among WLD-infected sugarcane fields where vectors were available naturally. 25x10m^2 size 6 plant plots were considered for the study. Sugarcane trash mulch was maintained in 3 plots and trash mulch was removed in 3 plots (un-mulched). Un-mulched three plots were maintained as control treatment. Vector populations in each plot were recorded in weekly intervals up to six months of age as, the number of vectors captured for 500 sweeps within the plot during ratoon I and ratoon II crops.

IV. Ovipositional preference of *D. menoni* in soil mulched with polythene

a. Laboratory study

Five mulches were used for the study. Polythene in four colours (Black, yellow, blue, transparent) and aluminum foil were used as mulches. Un mulched soil is considered as control. Three-month-old healthy sugarcane plants grown in plastic pots (14 x14cm) were obtained for the study. The plants were thoroughly cleaned by removing ants, spiders, and other insect predators before introducing treatments.

Ten plant pots from each mulch type were arranged by covering the soil with a layer of mulching material (polythene/ aluminum foil). Six pots from black, yellow, blue, transparent polythene, and aluminum foil enclosed in a single insect cage with un mulched plant pot for multiple choice test. Ten gravid females were introduced into the cage. Experimental setup was kept undisturbed for two week period for oviposition. The test setup was replicated ten times. After 2 weeks, polythene/aluminum foil was removed and the top soil layer in each pot was collected separately and examined through a light microscope (KYOWA TOKYO, 10x3) for the presence of eggs.

a. Field study

A field trial was established in Research Farm (SRI), Uda Walawe. The trial was located among WLD-infected sugarcane fields where vectors were available naturally. According to the results of the laboratory study on the effect of polythene mulch on the egg laying of vector, black colour polythene mulch was selected for the field test. Normal, un-mulched soil is considered as a control. 25x10m^2 size 6 plant plots were established with hot water-treated seed cane (SL 96 128) during the Maha season. After the germination of plants, the base of the plants in randomly selected three plots (both sides) were covered with 1.5' black polythene. Polythene mulch was fixed into the soil with wooden pegs to cover the soil properly at the base of the plants. The remaining three plots were maintained as control treatment. Vector populations in each plot were recorded in weekly intervals up to six month age as, the number of vectors captured for 500 sweeps within the plot.

Statistical Analysis

Data on egg counts were subjected to square root transformation, and the analysis of variance was conducted for the transformed data. Means were separated by Duncan's multiple range test at 0.05 probability level using the SAS software.

RESULTS AND DISCUSSION

I. Ovipositional preference of *D. menoni* in different soil types

a. Laboratory study

The highest number of eggs of *D. Menoni* was found in sandy loam soil followed by fine-sand soil. Similar observations of more eggs in sandy loam soil were found in all three experiments conducted in two seasons (*Maha* and *Yala*). There was no significant difference in number of eggs recorded between the two cropping seasons (Table 1).

Table 01: The mean number of eggs laid by ten *D. Menoni* (mean) in six soil types at fourteen days after introducing gravid females into rearing (laboratory) cage in the *Maha* 2014 and *Yala* 2015 seasons

Soil type	Average number of Eggs	
	Yala season	*Maha* season
Fine sand	5.66 [ab]	7 [ab]
Coarse sand	0 [b]	0 [b]
Sandy loam	20 [a]	23.25 [a]
Clay loam	0 [b]	0 [b]
Gravel	0 [b]	0 [b]
Clay	0 [b]	0 [b]

Note: In a column, means followed by common letters are not significantly different in 5% probability level.

D. menoni preferred sandy loam soil for laying eggs, as it had a significantly higher number of eggs. This could be attributed to the porous nature of the soil (Foth, 1978). Loose, porous soil facilitates the penetration of ovipositor into the soil for egg laying. The compacted soils restrict penetration. Also, poor ventilation in compact soil disturbs laying eggs and hatching (Simelane, 2007; Chaudarie, 1985). Yang and Pang (1979) have reported that, *Epitettix hiroglyphicus*; the vector of WLD in Taiwan, prefers fine sand, coarse sand and sandy loam soils for oviposition, and 10 %(w/w) was the most preferred soil moisture level for its oviposition.

b. Field study

According to a field survey conducted in farmers' fields in Sevanagala rain-fed sector, all highly WLD-infected fields were associated with sandy loam soils and 10% (w/w) moisture content (Table 02). The population levels of the vector were also comparatively high in those fields.

Table 02: Sand, clay, silt composition, soil textural class and vector population captured during 500 sweeps in each test field

Field No	Sand%	Clay%	Silt%	Texture class	Vectors
1	76.72	15.28	8.00	Sandy Lome	7
2	72.72	18.00	9.28	Sandy Lome	5
3	70.72	20.00	9.24	Sandy Lome	5
4	66.72	16.00	22.08	Sandy Lome	4
5	74.72	11.20	9.28	Sandy Lome	7
6	70.72	20.00	9.28	Sandy Lome	4
7	68.72	20.00	11.28	Sandy Lome	2
8	68.72	20.00	11.28	Sandy Lome	2
9	80.72	14.00	5.28	Sandy Lome	8
10	61.03	28.24	10.72	Sandy Clay Lome	1
11	70.72	22.24	7.04	Sandy Clay Lome	0
12	78.72	18.24	3.04	Sandy Lome	8
13	72.72	16.24	11.04	Sandy Lome	12

This confirms the finding of the laboratory study, the high preference of the vector for sandy loam soil for oviposition under favorable moisture levels. Hence, more attention should be given to managing WLD in the fields with sandy loam and fine sand soils in areas with high incidence of this disease.

ii. Ovipositional preference of *D. menoni* for soils incorporated with filter-mud and spent wash

a. Laboratory study

The number of eggs in filter-mud incorporated soil was 67.5% more than that in the unincorporated soils. The number of eggs laid in spent wash incorporated soil is significantly high compared to the spent wash unincorporated soil (Table 03).

Table 03: The mean number of eggs laid by three female adults of *D. menoni* fourteen days after introducing into the rearing cage

Treatment	Number of eggs (mean)
Filter mud	
Incorporated soil	8 [a]
Un-incorporated soil	2.6 [b]
Spent wash	
Incorporated soil	8 [a]
Un-incorporated soil	3 [b]

Note: In a column, means followed by common letters are not significantly different in 5% probability level.

a. Field study

High incidences of WLD vector have been recorded in the fields incorporated with filter-mud, spent wash, and both filter mud and spent wash incorporated fields than the filter-mud unincorporated fields (Table 04). Even though filter mud enhances soil quality by adding organic matter into the soil, this practice enhances the soil porosity facilitating penetration of ovipositor 1-2cm deep into the soil for laying eggs.

Table 04: Mean number of *D. menoni* adults captured during 500 sweeps in each plot with each treatment

Treatment	Number of insects (mean)
Filter mud-incorporated soil	8 [a]
Spent wash incorporated soil	7.5 [a]
Filter mud and spent wash incorporated soil	9 [a]
Control/Un-incorporated soil	3.5 [b]

Note: In a column, means followed by common letters are not significantly different in 5% probability level.

iii. Ovipositional preference of *D. menoni* for the soil mulched with sugarcane trash

In the laboratory study, the mean number of eggs in the soil mulched with sugarcane trash was slightly higher (5.4[a]) than that in un-mulched soil (5.2[a]), but the difference was not statistically significant. Also, according to the data collected from the field study, the number of vectors in the fields with sugarcane trash was slightly higher (8.9[a]) than that in the fields without sugarcane trash (8.4[a]), but the differences were statistically not significant. However, *D. menoni* occasionally lays eggs on sugarcane trash (Senevirathne, 2008).

Howard and Oropeza (1998) have discovered that different types of mulching materials have influenced on the abundance of the vector (*Myndus crudus*) of coconut lethal yellow disease and very few nymphs have been present near the coconut trees that have been mulched with coarse materials. Douglas and McClure (1988) have designed an approach to overcome the “X-disease of stone fruit” by manipulating the vector population by maintaining bare ground under the fruit crop. But this study reveals that there are no significant effects of trash on the vector population. It proves that removing or burning trash does not reduce the population level of WLD vectors in sugarcane fields.

iv. Ovipositional preference of *D. menoni* in soil mulched with polythene

a. Laboratory study

The lowest number of eggs was recorded in Black and transparent polythene mulched plant pots. A significantly high amount of eggs was recorded on pots with un-mulched soil (Table 05). There is no significant difference between black colour polythene mulch, transparent polythene mulch and aluminum foil mulch. Since aluminium foil is costly and transparent polythene degrade easily, black colour polythene was selected for the field study.

Table 05: The mean number of eggs laid by three *D. menoni* females on six soil mulches at fourteen days after introducing gravid females into rearing (laboratory) cage

Mulch type	The average number of Eggs
Soil (control)	7 [a]
Blue colour polythene	3 [ab]
Yellow colour polythene	2.33[ab]
Black colour polythene	0.33[b]
Transparent polythene	0.33[b]
Aluminium foil	0.66[b]

Note: In a column, means followed by common letters are not significantly different in 5% probability level.

a. Field study

Table 06: Mean number of *D. menoni* adults captured during 500 sweeps in each age of the black colour polythene mulched and un-mulched plots

Crop age	The mean number of vectors / 500 sweeps	
	Polythene mulched	Un mulched soil
1st Month	0	0
2nd Month	2[a]	2.2[a]
3rd Month	3.33[a]	3.5[a]
4th Month	7[a]	7[a]
5th Month	3.5[a]	3.33[a]
6th Month	0.75[a]	0.8[a]

Note: In a column, means followed by common letters are not significantly different in 5% probability level.

As the number of eggs in the soil is not detectable, the number of insect vectors caught in the insect net is considered a relative measurement of eggs. There was no significant difference recorded in the mean number of *D. menoni* adults captured in polythene mulched and un-mulched plant plots (Table 06). Accordingly, no significant effect of black polythene mulch was recorded under field conditions.

Since it is not possible to cover the soil completely under field conditions, using polythene mulch to deduct the oviposition rate of the vector is not an effective vector management strategy. The ridge and furrow land preparation system for sugarcane is also may be a reason for the ineffectiveness of the mulch. Because due to irregular land proper mulching/ covering soil is difficult. Tillering nature of the plant also may cause a reason for the incompatibility of mulch to the soil. However, the effect of the polythene mulch in ratoon one crop will be tested with the ratoon crop of the same plant crop used in the study.

CONCLUSIONS

There is a significant difference in ovipositional preference of the WLD vector *D. Menoni* between different soil groups and between different soil amendments. There is a high preference of *D. menoni* for oviposition in sandy loam and fine-sand soils. Therefore, more attention should be given to managing WLD in the fields with sandy loam and fine-sand soils in the areas with a high incidence of this disease. This could be accomplished by adopting recommendations given by SRI on the use of healthy seed cane, regular monitoring of the disease incidence in the field, and regular roguing out of the infected plants at the early stage. Filter mud was found to be a more preferred substrate for oviposition by *D. menoni*. This has to be considered in using filter mud to enhance the soil quality, particularly in WLD susceptible areas having a high vector population. The above investigations were conducted only at Uda Walawe under controlled conditions and a limited number of seasons, sites, and locations. The results reported are very useful as baseline information for developing a WLD management program, and further studies are required to confirm the applicability of findings under different field conditions.

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Best Time for Application of Insecticides for Controlling *Deltocephalus menoni* (Hemiptera: Cicadellidae), a Vector of Sugarcane White Leaf Disease in Sri Lanka

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ABSTRACT

White Leaf Disease in sugarcane caused by a phytoplasma, which is transmitted by Deltocephalus menoni (Hemiptera: Cicadellidae), has become a severe threat to the sugarcane industry in Sri Lanka. Therefore, monitoring of vector populations and the application of insecticides are required to contain secondary transmission of the disease. This study aims at studying the feeding calendar of D. menoni during 24 hours of a day to determine the best time for population studies and insecticide application. The study was conducted at the Entomology laboratory of the Sugarcane Research Institute, Sri Lanka, using insect-feeding chambers fixed to the top leaf of 4-month-old sugarcane plants of the variety SL 96 128. Water-starved 150 insects; fifty from each nymph and male and female adults were inserted individually into the feeding chamber. The area stained due to the honeydew excreted by the insect was measured hourly using the Bromocresol-treated filter papers, and its variation was analysed by the Analysis of Variance and mean separation by Least Significant Difference at 5% probability. The results revealed that both the nymphs and the adult males and females showed a similar pattern of honeydew secretion, and hence, their feeding. The excretion of honeydew was significantly higher from 6.00 am to 9.00 am and 4.00 pm to 7.00 pm than that at other times of a day. Population studies and insecticide application for controlling of D. menoni should be carried out during these time periods for more efficient results.

Keywords: *Deltocephalus menoni,* Population studies, Sri Lanka, Sugarcane white leaf disease, Vector

INTRODUCTION

White Leaf Disease in sugarcane (WLD) caused by phytoplasma has become devastating with significant losses in both cane yield and sugar content in cane, and hence, a serious threat to the sustenance of the sugar industry in Sri Lanka. The causal organism of WLD transmits primarily through infected seed cane and secondarily by the insect vector, *Deltocephalus menoni* (Hemiptera: Cicadellidae). Knowing the precise population and time for application of insecticides to control WLD-transmitting vector has become a necessity to contain the further spread of the disease in sugarcane plantations. Both the nymphs and the adults of *D. menoni* feed on phloem sap of sugarcane leaves. *D. menoni* spends the daytime within the soil or occasionally on the leaf sheaths at the base of the plants and on sugarcane trash (Senevirathne, 2008). It comes out of their hiding places to the canopy for feeding, and therefore, it is the best time to kill a higher number of the vector by insecticide application. Knowledge of the pattern of feeding on a day by the vector on leaves is required to determine the best times for undertaking population studies and application of insecticides for its more efficient control. The present study was conducted to identify the feeding calendar of *D. menoni to* determine the best time periods for undertaking population studies and application of insecticides on *D. menoni* by identifying the time of the day when the vector is feeding on sugarcane leaves, for its efficient control.

MATERIALS AND METHODS

The study was conducted in the Entomology laboratory of the Sugarcane Research Institute (SRI), Uda Walawe, Sri Lanka, from October to December 2016 using four-month-old sugarcane plants of the variety SL 96 128 produced through meristem culture. *D. menoni* was reared according to the methods developed by Senevirathne, (2008). A total of 150 insects, fifty from each nymph and adult males and females (2 days after ecdysis) were separately used for the test. The test insects were water starved for 3 hours before placing them in the feeding chamber prepared with transparent sheets with dimensions 5x3x5 cm, which were attached to the top leaf of each test plant. Twenty-four Bromocresol green-treated filter paper strips with 3 cm width and 5cm length, attached, were fixed at the bottom of the feeding chamber to measure the area stained due to honeydew excreted by *D. menoni*. This arrangement is depicted in Figure 1.

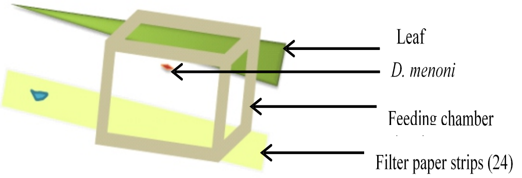

Figure: 1 Feeding chamber set up for measuring excreted honeydew by *D. menoni*

The water-starved insects were introduced individually into the feeding chamber and allowed to feed on sugarcane leaves without any disturbance. The filter paper strip in the chamber was slowly drawn out in a way that the next portion of the strip moves into the chamber, one hour after an introduction and at one-hour intervals. After 24 hours, honey-dew-stained filter paper strips were collected and air-dried. Honey dew-stained area on the paper for each hour was measured with a transparent 1 mm^2 grid. The variations in honey dew-stained areas for nymphs and male and female adults were analysed by the Analysis of Variance (ANOVA) and mean separation was done using Least Significant Difference (LSD) at the 5% probability level.

RESULTS AND DISCUSSION

The nymphs and both male and female adults showed a similar pattern of honeydew production at different times of a day (Fig 2). The highest honeydew production was recorded during the 4.00 to 5.00 pm (9.6 to 10.08 mm^2) during the day. The honeydew production was comparatively high from 6.00 am to 9.00 am and 4.00 pm to 7.00 pm.

A similar pattern of feeding by several leafhopper species has been reported elsewhere. In a study on grape leafhopper (*Erythroneura elegantula)* Kido and Stafford (1965) found that the number of droppings increased during the morning hours (6.00 to 9.00 am), reached a peak, and then decreased in the early afternoon. This decline was followed by an increase in the late afternoon or early evening (3.00 to 6.00 pm). Naito (1977) has confirmed that the stylet insertion behaviour of leafhoppers shows apparent diurnal fluctuations. According to the observations on the performance of *Nepholeltix cincliceps* on rice leaves, the insects behave vigorously, repeating the insertion successively in the evening (5.00 to 9.00 pm). After 10.00 pm *N. cincliceps* rest, and in the early morning, 5.00 to 6.00 am, the frequency of insertion is slightly high. He has also confirmed that the leafhopper population in the canopy has a significant positive correlation with

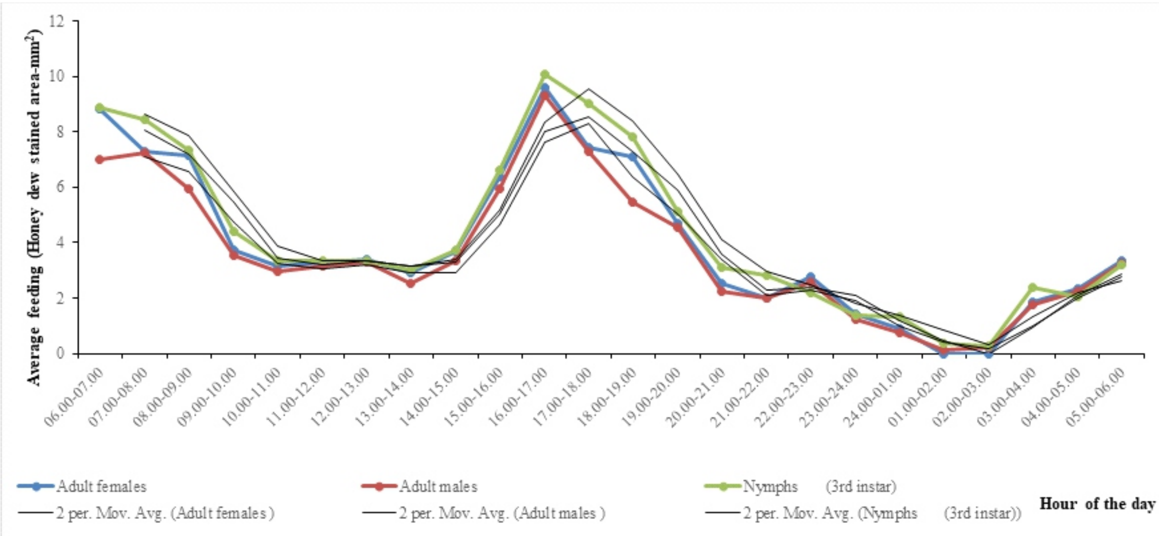

Figure 2: Average honeydew excretion by nymphs and male and female adults of *D. menoni* in an hour during 24 hours

relative humidity at 8.00 am and an increase in the rate of feeding at low temperatures and a decrease at high temperatures. Similarly, the current study has also shown a slower rate of feeding during the daytime (9.00 am to 4.00 pm) due to high temperature (< 33.7 °C) than that in the morning (22.3 °C) and in the evening (22.5 °C) when the temperature is low. In this study, it has been observed that the rate of honeydew excretion by the nymphs of *D. menoni* was higher than that of the adults of which the females excrete honeydew at a higher rate than the males.

CONCLUSIONS

Nymphs, male and female adults of *D. menoni* showed a similar feeding pattern in terms of the amount of honeydew secretion at different times of the day by feeding on sugarcane leaves. The peak feeding times found in this study are 6.00 to 9.00 am in the morning and 4.00 to 7.00 pm in the evening. Therefore, the above time periods are the best times for population studies and the application of insecticides for increasing the efficiency of controlling *D. Menoni* population to control white leaf disease in sugarcane.

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Production of Silage Using Sugarcane Tops and Testing Nutritional Quality

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ABSTRACT

Shortage of good quality cattle feed is one of the main problems in the dairy industry in the Dry Zone of Sri Lanka and it is further worsened during the dry season. Hence, the objectives of this experiment were to: (a) produce quality improved cattle feed using sugarcane tops as an alternative to the fodder of Guinea "A" (Panicum maximum) and (b) evaluate ensiling characteristics and nutritive value of preserved sugarcane tops based cattle feed. Sugarcane tops and Guinea grass were preserved by mixing rice bran, coconut poonac, molasses, urea, and minerals, and silage were prepared. Physical characteristics and nutritional quality of silage were analysed and compared with Guinea grass. Sugarcane tops silage had an olive green colour, fruity aroma, moist texture, and good fermentation characteristics with a low pH value (4.5 - 6.2). Adding urea or urea with rice bran increased crude protein in sugarcane tops silage. The lactic acid content ranged from 14.8% to 15.4% in sugarcane tops silage. The silage samples made with only sugarcane tops recorded significantly high soluble carbohydrate content than Guinea grass silage. Ensiling sugarcane tops with molasses, rice bran, urea, and coconut poonac could produce nutritive silage for feeding cattle in sugarcane growing areas of Sri Lanka.

Key words: Cattle feed, Guinea grass, Silage, Sugarcane tops

INTRODUCTION

Lack of good quality feed year round is a major constraint to profitable smallholder dairy production in Sri Lanka (Ibrahim *et al.*, 1999*)*. Most of cattle are reared in Dry Zone traditional Village System where indigenous cattle are maintained on common grazing lands with minimal inputs (Abeygunawardena *et al*., 1997). As low returns from the low yielding indigenous cattle, farmers in Dry Zone of Sri Lanka reluctant to feed cattle on concentrates. Direct feeding of fresh fodder and Guinea grass is limited to the shorter wet season in Dry Zone. There is a little scope for feeding cattle using hay in the dry season, but it is not popular due to its low nutrient content and less palatability (Preston, 1977). Therefore, the introduction of good quality animal feed produced from locally available fodder would enhance the productivity and profitability of the dairy industry in the Dry Zone of Sri Lanka. The preservation of fodder by fermenting with molasses (silage) increases the nutrient contents and the palatability of the fodder (Stewart, 2011). The unavailability of enough fodder materials, particularly in the dry season in the Dry Zone of Sri Lanka, restricts the production of silage. Sugarcane cultivation which is the main livelihood of rural communities in Monaragala and Ampara districts, is harvested mainly during the dry season (Mettananda, 1990). Sugarcane plantation of 10,000 ha produces about 50,000 - 75,000 tons per annum (Anonymous 2013). Kirk and Crown (1942) reported that (sugarcane tops) SCT could be fed to cattle as fresh and ensiled materials. In addition, the integration of cattle with sugarcane cultivation improves productivity as a more sustainable system of farming (Smith *et al*., 1997). However, farmers in the Dry Zone of Sri Lanka do not pay enough attention to the integration of sugarcane cultivation and cattle management. They do not get a substantial benefit from using SCT as a cattle food, neither fresh nor ensiled forms though it has a nutritional value than rice and wheat straw (Preston, 1977). Ensiling and preservation further improve the nutritive value and the palatability of the SCT (Bui Van Chinh *et al*., 2000). Therefore, a study was conducted to produce silage using SCT and testing the nutritive values of prepared SCT silage.

METHODOLOGY

Production of SCT silage was done at the research farm, Sugarcane Research Institute (SRI), Udawalawe. The laboratory analysis was conducted at the Veterinary Research Institute (VRI), Gannoruwa, Peradeniya, and the Department of Animal Science, Faculty of Agriculture, University of Peradeniya, to investigate the ensiling characteristics of green cane tops. The sugarcane tops of 12 months age crop of the variety Co 775 cultivated under rain-fed condition at the research farm of SRI was used for the study. Guinea grass (*Panicum maximum*) collected from the research farm was used as the control treatment. The experimental design was Completely Randomized Design (CRD) with eight treatments (Table 1) and three replicates. Two types of green fodders (SCTs and Guinea grass) and four levels of additives mixed into SCT and Guinea grass were tested.

Table 01: Treatments used for the study

Treatment	Percentage of ingredients used				
	SCT	Guinea grass	Urea	Rice bran	Molasses
T1	100	-	-	-	-
T2	99	-	1	-	-
T3	96.5	-	1	2.5	-
T4	98	-	-	-	2
T5	-	100	-	-	-
T6	-	99	1	-	-
T7	-	96.5	1	2.5	-
T8	-	98	-	-	2

Note SCT: sugarcane tops

Silage preparation

Chopped sugarcane tops and Guinea grass samples were ensiled with additives, as mentioned in Table 01. Then, the mixtures were filled into transparent polyethylene bags (lab silos), pressed to remove trapped air, sealed, and stored for up to 35 days for ensiling. The silos were put into black colour polyethylene bags to prevent the exposition of light and stored at room temperature.

Laboratory analysis of silage samples

After 35 days, silos were opened, and visual observation was made for colour, odour, texture, and presence or absence of mould. Water extracts of the ensiled mixtures were used to measure the pH value of each treatment. For this, 25 g of sample mixing with 225 ml of distilled water was blended using a blender, and the mixture was filtered using filter paper. Then, the pH of the filtrate was measured immediately using a pH meter. Dry matter (DM) contents of the silage samples were determined by drying in an oven at 60 °C to a constant weight. Water-soluble carbohydrate (SC) content was determined using the Anthrone test (AFIA, 2011). Lactic acid (LA) and ammonia nitrogen (AN) were analysed using a spectrophotometer according to the Barnett (1951) and Parsons *et al.* (1984) methods, respectively.

RESULTS AND DISCUSSION

Physical characteristics of silage

Table 02 presents the colour of silage samples after four weeks from the date of preparation. The T1 (SCT only) had an olive green colour, and T5 (Guinea grass only) had a green colour. The silage prepared by adding only urea (T2) was darker than the silage prepared with urea and rice bran (T3). The T1 and the T4 (SCT+ Molasses) had a pleasant fruity odour compared to T2 and T3. Guinea grass ensiled with urea (T6) had a slightly bad smell, but it was not spoilt. This conformed to the results reported by Tuah *et al.,* (1979). The uniformity and texture of all the samples were at a satisfactory level, and they were free from moulds. Overall acceptability of all the silage samples prepared with SCT was better than that of Guinea grass silage.

Nutritional values of silage

Table 02 describes the nutritional value of SCT silage and guinea grass silage samples compared to different treatments with DM, CP, CF, pH, LA, AN, and SC content.

Dry matter content

The DM content of silage samples ranged from 33.88% to 35.84% (Table 02). The DM content of SCT silage was not significantly changed due to the addition of urea, rice bran, and molasses. However, the addition of 2% molasses to Guinea grass significantly increased the DM content ($P<0.05$). According to McDonald (1981), the addition of molasses caused to addition of more soluble carbohydrates for lactic acid bacteria (LAB) and prevented the breakdown of sugars and organic acids in the grass and may have reduced the DM losses. Also, the addition of rice bran to SCT did not increase the DM content. This may be due to the loss of DM during the fermentation process.

Crude protein

The addition of urea at the time of ensiling significantly ($P<0.05$) increased the crude protein content of the silage. However, the crude protein content of the silage did not change on the type of forage (Table 02). For SCT ensiled with urea and rice bran (T2, and T3) had higher crude protein content than T1 (Only SCT). Also, Guinea grass ensiled with urea and rice bran (T6 and T7) had higher crude protein content than T5 (Only Guinea grass). According to Alcantara *et al.* (1989), the addition of urea increased the pH of ensiled forages, and it had shown positive effects in the preservation of nutrients in SCT silage. Several types of research have shown that SCT ensiled with urea remarkably increased its crude protein content (Noroozy and Alemzadeh, 2006a; 2006b; Alemayehu *et al*., 2014). Rangnekar (1988) reported that SCT silage ensiled with 0.5% urea contained 8.1% crude protein. In the present study, crude protein content in silage prepared with 1% urea was 12.72%. This may be due to the differences in urea percentage used, sugarcane crop maturity, variety, and climatic condition of the sugarcane growing area. However, the addition of molasses when preparing SCT silage increases the crude protein content. According to McDonald (1981), the addition of molasses increases the soluble carbohydrates, and it can act as a substrate for LAB to produce lactic acid, which will decrease the pH. Therefore, molasses reduced the breakdown of protein and nitrogenous compounds in forage samples, and this resulted in the preservation of nutrients during ensiling (Carpintero *et al*., 1969).

pH value of silage

The pH value of silage prepared from only SCT (T1) was significantly lower ($P<0.05$) compared to Guinea grass silage (T5) (Table 02). Ensiling both sugarcane tops and Guinea grass with urea or urea with rice bran significantly ($P<0.05$) increased the pH compared to other treatments (Table 02). According to the literature, SCT ensiled for 45 days with 2% molasses had recorded a pH value of 4.8 (Bui Van Chinh *et al*., 2000). In this study, the lowest pH was recorded when SCT are ensiled with 2% molasses (T4). Because SCT contains more soluble sugars than the other type of grasses, and fermentation process may take place more efficiently. Preston *et.al*, (1976) reported that most forages present complications in ensuring adequate fermentation when they are ensiled due to low soluble sugar content. Therefore, molasses has to be added.

Water soluble carbohydrate content

Table 02 presents the effect of different additives on the water-soluble carbohydrate content of silages. The water-soluble carbohydrate content of the silage of both SCT and Guinea grass increased with the addition of molasses (Table 02). However, the silage made from only SCT (T1) had a significantly high ($P < 0.05$) water-soluble carbohydrate content than the silage made with only Guinea grass (T5) (Table 02). This may be due to the high concentration of total sugars in SCT (Singh and Solomon, 1995). Water-soluble carbohydrates are important for the LAB as a substrate during the ensiling process (McDonald, 1981). According to Pate (1981), sugarcane leaves are also rich in soluble carbohydrates. Therefore, SCT are good forage for the ensiling process. In general, tropical forages are low in water-soluble carbohydrates (Ibrahim *et al*., 1989). Moreover, Catchpole and Hanzel (1971) reported that tropical grasses contain relatively high concentrations of cell wall components and a low level of fermentable carbohydrates. This might be the reason for differences in the chemical properties of silage made out of Guinea grass than SCT.

Lactic acid concentration

In the present study, the lactic acid concentration of SCT and Guinea grass silage ranged from 14.71% to 15.58% (Table 02). Significantly high ($P<0.05$) lactic acid concentrations were recorded in the silage samples prepared with SCT mixed with rice bran and molasses compared to the same treatment prepared with Guinea grass. Lactic acid concentrations in silage samples prepared using only SCT or Guinea grass were significantly high ($P<0.05$) when it was prepared by adding only molasses (Table 02). However, Suzuki (2014) reported that lactic acid content was 1.08% for SCT silage made from 6 months of age SCT without additives. Sudarshan (2000) reported that lactic acid content was 5.24% for ensiled Guinea grass with 5% molasses. The lactic acid concentrations of the silage produced in the present study were higher compared to the above findings.

Table 02: Fermentation qualities and color of sugarcane tops and Guinea grass silage

T	DM (%)	CP (%)	pH	LA (%)	AN (%)	SC (%)	C
T1	34.82 [ab]	4.40 [e]	4.66 [d]	14.77 [cd]	1.35 [e]	7.34 [c]	Olive green
T2	33.88 [b]	12.72 [b]	6.20 [b]	14.76 [cd]	9.97 [b]	5.60[d]	Darker than T1
T3	34.06 [b]	16.02 [a]	6.16 [b]	15.35 [ab]	10.14 [ab]	4.69 [e]	Darker than T4
T4	34.52 [b]	5.38 [d]	4.59 [d]	15.22 [abc]	1.67 [e]	11.59 [a]	Brown green
T5	34.26 [b]	8.03 [c]	5.73 [bc]	14.99 [bcd]	4.98 [c]	6.31 [d]	Green
T6	34.21 [b]	13.29 [b]	8.64 [a]	14.71 [d]	10.43 [a]	7.86 [c]	Darker than T5
T7	35.13 [ab]	15.59 [a]	9.01 [a]	14.76 [cd]	9.82 [b]	7.27 [c]	Darker than T8
T8	35.84 [a]	8.82 [c]	5.24 [c]	15.58 [a]	3.11 [d]	8.90[b]	Brown green
CV %	2.01	5.19	4.71	1.68	3.23	6.56	

Note T: Treatment, DM: Dry Matter, CP: Crude Protein, LA: Lactic Acid, AN: Ammonium Nitrogen, SC: Soluble Carbohydrates, C: Colour

*Means within a column with the same letters are not significantly different ($P>0.05$)

Ammonia nitrogen level

Ammonia nitrogen levels of silage made with urea or urea with rice bran were significantly higher ($P<0.05$) than those silage prepared with or without molasses, regardless of the type of forage (Table 02). Ammonia nitrogen is a good indicator of the quality of silage, and it is closely related to the silage pH (Carpintero *et al*,, 1969). According to Chaudhary *et al*,. (2012), tropical forage silage of good quality had a pH of less than 5.0 and less than 15% of ammonia nitrogen of the total dry matter. Ammonia nitrogen levels in all silages prepared in the present study were in agreement with the results of Chaudhary *et al*, (2012). In general, 5% ammonia nitrogen content is excellent, whereas 5 - 10 % is good in quality. Therefore, the silage prepared with only SCT or only guinea grass and the silage prepared with SCT + molasses in the present study were excellent in quality (Table 02).

CONCLUSION

Fermentation characteristics of silage depend on the type of forage and the additives mixed during silage preparation. The addition of molasses has a positive effect, whereas the inclusion of urea leads to develop a negative effect on the fermentation characteristics of silage. According to this study, sugarcane tops can improve the fermentation characteristics in the silage-making process compared to the guinea grass silage, and the addition of molasses can enhance the fermentation characteristics of silage. Therefore, sugarcane tops can be recommended as an optional feed material for making good-quality silage under local conditions.

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Effects of Sugarcane-byproduct, Vinasse on Chemical Properties of Soil and Initial Growth of Sugarcane Varieties SL 83 06 and SL 96 128

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ABSTRACT

Vinasse is an aqueous effluent of the distillation unit in the sugar-alcohol industry and a problem to the sector due to its potential effects as an environmental pollutant. However, proper usage of vinasse contributes to improving soil quality and agricultural productivity. The objectives of this study were to evaluate the effects of sugarcane vinasse on soil chemical properties and initial growth attributes of the sugarcane plant. The research consisted of a laboratory and a pot experiment. In the laboratory experiment, concentrated vinasse (volume 1:10) was applied to soil at the level of 40 m^3/ha, and non-concentrated vinasse was applied to soil in four levels; *viz.* 40, 60, 80, and 120 m3/ha to evaluate soil chemical properties (pH, electrical conductivity, organic matter, nitrogen. phosphorus, and potassium). Data were collected up to for 98 days in the laboratory experiment. Similar treatments were applied for soil pot culture-grown varieties, SL 83 06 and SL 96 128 as the above ratios of concentrated and non-concentrated vinasse under net house condition. The results of the laboratory experiment indicated that the concentrated vinasse-treated soil samples showed considerably higher values for all tested chemical properties except soil pH. Both varieties had performed well in 40 m^3/ha non-concentrated vinasse level. However, the variety, SL 83 06 showed higher performance than SL 96 128 in shoot dry weight, root length, shoot dry weight, and root dry weight at 40 m^3/ha non-concentrated vinasse level. Since, findings indicated that, lower doses of non-concentrated vinasse are more favorable to plant growth with a stimulatory effect on plant initial growth.

Keywords: Concentrated and non-concentrated vinasse, soil chemical properties, Sugarcane

INTRODUCTION

Recently, the high cost of fertilizers and concerns about environmental protection have been great incentives to study the recycling of the large quantities of organic residues produced as byproducts of the sugar and alcohol agro-industries in agriculture. Vinasse is an aqueous effluent of the distillation process in the sugar-alcohol industry. Disposal of vinasse has become an acute problem for the sector due to the large quantities produced and its potential effects as an environmental pollutant. The composition of vinasse varies, but in general, it is composed mainly of water, organic matter, and mineral elements and characterized by undesirable colour, foul odour high biological oxygen demand (BOD) chemical oxygen demand (COD). It contains many useful elements and can be used as a dilute organic liquid fertilizer to improve soil properties and increase crop yield while alleviating environmental pollution (Pande *et al*., 1995).

The quantity of vinasse produced depends on the processing technique employed and varies between 10 and 18 litres of vinasse per litre of alcohol produced. (Silva *et al.*, 2007). The disposal of this residue represents a major environmental concern, mainly due to the vast amount of wastewater (about 97%) and high organic loads. The environmental damage caused by discarding vinasse into the soil or running water was an incentive for studies aiming to find alternative, economic applications for this residue. Thus, its application in the soil is one of the most cost-effective and efficient ways of utilization, leading to improvement in the physical and chemical characteristics of soil and increasing crop yield. (Canellas *et al.,* 2003; Tang *et al.*, 2006). Results from such studies indicate that properly used vinasse contributes to improvements in soil quality (Ou *et al.*, 2002) and agricultural productivity (Li *et al.*, 2007 and Shang *et al.*, 2013). There have been some records about the effects on soil properties including physical, chemical, and microbial aspects (Singh *et al.*, 2008). However, more evidence is needed to illustrate and authenticate the mechanisms of vinasse application on the promotion of plant growth.

Organic matter, K, N, Ca, and Mg are the main chemical components of vinasse, K being the most important mineral element for the agricultural use of the residue. It is possible to estimate the potential contribution of vinasse for the annual recycling of nitrogen, phosphorus, and potassium in cultivated land with sugarcane. Gomez (1996) stated that, in three years of field trials, the application of vinasse increased sugarcane yield significantly without reducing quality further he also suggested that vinasse could substitute for 55% N, 72% P, and 100% of K required to sugarcane in Venezuela. Further, vinasse is used by sugar mills in Brazil as a fertilizer. It can replace, in part or full, potassium fertilizers for sugarcane (Su *et al.*, 2009), representing an economical alternative. So effective usage of vinasse provides the best option for reducing the cost of production and increasing profit in sugar industries.

Nevertheless, for sugar mills with limitations to dispose of vinasse in the soil, one solution would be to transport it to distant locations. Nevertheless, this would result in a cost increase due to a large amount of water waste. Under such a scenario, as an economical alternative for transportation, the volume of vinnase has been reduced by evaporation to obtain the byproduct with lower water content. The non-concentrated and concentrated vinasse have different compositions, water content, and mineralization pathways (Parnaudeau *et al.*, 2006). The concentrated vinasse by evaporation is increasingly used by sugarcane mills in Brazil. However, the amount of vinasse applied in agriculture must follow appropriate guidelines, which vary according to the soil characteristics. In Sri Lankan scenarios, there is an already established recommendation on direct soil application of vinnase to sugarcane fields. However, it is required to monitor and study furthermore on this matter further for upcoming problems related to vinasse in sugarcane-growing soils. A specific recommendation must be followed for each condition to prevent excessive use and consequent mineral lixiviation. Therefore, this study aimed to evaluate the effects of sugarcane vinasse (non-concentrated and concentrated) on soil-chemical properties and initial growth attributes of sugarcane varieties SL 83 06 and SL 96 128

MATERIALS AND METHODS

Initial Sample Collection

Soil samples were collected from the research farm of the Sugarcane Research Institute in 2015. Soil is Reddish Brown Earth and it had the following chemical characteristics; pH: 6.6, EC: 2.6 ms/cm, N: 800 ppm, P: 29 ppm, K: 190 ppm, and OM: 7.5 %.

The non-concentrated vinasse (NCV) used in the experiment was obtained from the sugar mill distillery, Sevenagala with a high content of water. The concentrated vinasse (CV) was obtained by the evaporation process of the NCV, concentrated ten times its volume. The chemical composition of NCV and CV are shown in Table 1.

Laboratory experiment

In the laboratory experiment, the treatments consisted of one dose of CV (40 m^3/ha) and four levels of NCV (40 m^3/ha, 60 m^3/ha, 80 m^3/ha, 120 m3/ha) including a control (without vinasse application). The above-mentioned levels of vinasse were incorporated in plastic bottles with 100 g of soil collected from the research farm and kept at room temperature. Soil moisture content was maintained at 70% field capacity in all the treatments by adding distilled water. Since CV was highly viscous, it was diluted with the same amount of distilled water used to increase moisture to field capacity. The samples were analyzed 7, 14, 28, 42, 70, and 98 days after treatment allocation to evaluate soil chemical properties (pH, electrical conductivity, organic matter, nitrogen. phosphorus, and potassium).

Pot experiment

Single bud setts of sugarcane (*Saccharum spp.hybrid*) varieties SL 83 06 and SL 96 128 were grown in pot culture conditions containing soils collected from the research farm (pH 6-7) with laboratory-tested levels of NCV and CV with control (Water treatment). The pots were kept in net house condition, and growth attributes (shoot length, root length, shoot dry weight, root dry weight) were recorded for 30 days after planting.

Data analysis

In both experiments, Standard deviation (+SD) was calculated using means of three replicates as described by Panse and Sukhatme (1985). As per the collected growth attributes data shoot length index, root length index, shoot dry weight index, and root dry weight index were calculated as follows.

$$\text{Index Value} = \frac{\text{Value of the growth attribute}}{\text{Value of the growth attribute of control treatment}} \times 100\%$$

RESULTS AND DISCUSSION

Chemical composition of non-concentrated and concentrated vinasse

Chemical composition of vinasse varies considerably from one distillery to another depending on the raw material used in fermentation, the type and efficiency of fermentation, distillation, and the varieties and the degree of ripeness of the cane used. (Mary et al., 1996). NCV and CV used for the study were analyzed for some chemical parameters (Table 1).

Table 1: Chemical composition of non-concentrated and concentrated vinasse

waste	pH	EC mS/cm	Organic carbon	Nitrogen (mg/L)	Phosphorous (mg /L)	Potassium% (mg /L)
NCV	4.5	39.7	3%	1204	320	6000
CV	5.3	78.4	9%	2789	624	44000

Effects of sugarcane vinasse on chemical properties of soil

Figure 1-5 presents variations of pH, EC, OM, N, P, and K in soil treated with different levels of NCV and CV with the time in laboratory conditions. The results of the laboratory experiment indicated that the CV-treated soil samples showed higher values for all tested chemical properties except soil pH.

Concerning the pH, it was observed that the application of NCV with increasing rates to the investigated soil had a slight effect on soil pH. Conversely, when CV is applied soil becomes more acidic than NCV applied to the soil. pH values of NCV-applied soil range from 7.2-7.6 (Figure 1). Even though NCV is an acid residue with pH 4-4.5, the soil buffers its pH value within a range of neutral which is favorable for plant growth.The effect of CV on pH may be explained by the production of organic acids and hydrogen ions (H+). The decomposition process accelerates the release of CO_2 and organic acids that would reduce soil pH. This finding confirms those obtained by EI-Leboudi *et al.,* (1988) and Arafat (1994). The optimum soil pH for sugarcane growth is about 5.5 - 6.5 level and vinnase application has not adversely affected the optimum soil pH. Data also show that the Electrical conductivity (EC) values of the CV and NCV treated soil increased with the increasing rates of vinasse application, but no adverse effect on sugarcane plant growth. pH and EC levels did not show any large variation with the time.

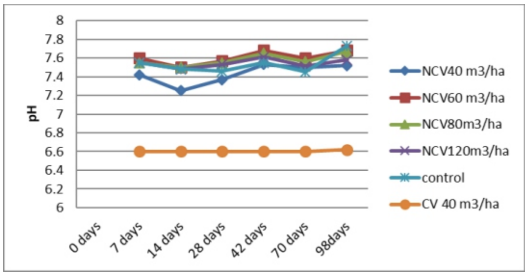

Figure 1: Variation of soil pH values in different levels of NCV and CV with the time in laboratory .

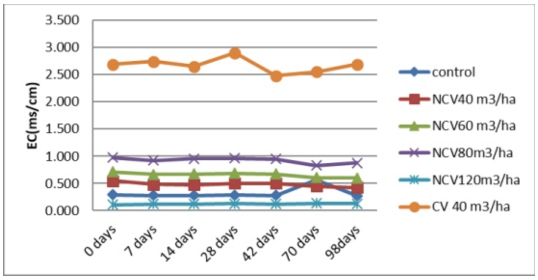

Figure 2: Variation of soil EC values in different levels of NCV and CV with the time in laboratory conditions.

Also, vinnase application increased the soil N, P, K, and organic matter levels with increasing rates of vinasse applied. The highest N, P, and K levels were observed in CV 40 m3/ha applied to the soil, and the highest OM content was in NCV 120 m3/ha level. According to Rossetto *et al.*, (2008) stated that in general vinasse presents a high content of organic matter and potassium. Thus its application in the soil is one of the most cost-effective and efficient ways of utilization of sugar alcohol industry effluent.

The most striking change was the tremendous increase in soil OM, N, and K content, as the result of treating the soil with CV. The percentage increase in the organic matter was more than 50% at the rate of CV 40 m^3/ha relative to the control (Figure 3). Similar results were obtained by Orlando Fillo (1996), who stated that the addition of vinasse to soil led to an increase in the amount of organic matter and K content.

It was observed from Figures 4, 5, and 6 that the extractable concentration of N, P, and K in soil treated with CV increased relative to NCV. The rate of increase depends mainly on the rate of vinasse applied. The magnitude variation of residual extractable potassium was observed with respect to the rate of vinasse applied. The highest values of N, P, and K were observed in CV-treated soils. Phosphorus and potassium contents gradually increased with time except for nitrogen. Conversely, the N level in NCV-treated soil has decreased with time. However, the N level in CV-treated soil has gradually increased at a slow rate and time. The above results further authenticate the findings of Alinne *et al.,* (2013), as they stated that the NCV is a good alternative to be applied as a soil nutrient source. However, higher doses promote N losses by denitrification due to high water content.

N mineralization and availability of nitrogen highly depend on the water content, aeration, quantity, and nature of organic matter added to the soil by CV and NCV and their doses, which produced distinct effects on the indigenous N, once the N addition affects the nitrogen transformation in the soil (Kuzyakov *et al*., 2000).

According to Alinne *et al.,* 2013 he stated the NCV is a good alternative to be applied for sugarcane crops but promotes N losses by denitrification due to high water content and it is also leaching losses.

The highest P and K levels observed in the CV 40m^3/ha ratio which is gradually increased with time. As with the vinnase application, P content was increased from 81 to 118 ppm and K content was increased from 800 to about 1200 ppm. These results indicate that the P and K levels in the soil were positively affected by the rate of vinasse applied to the soil (Figures 5 and 6). These results are in good agreement with those obtained by Gomez (1996) and Orlando (1996) and they stated that the application of vinasse could provide added nutrients to sugarcane, similar to mineral fertilizers application, besides the benefits of organic matter and micronutrients addition to the soil.

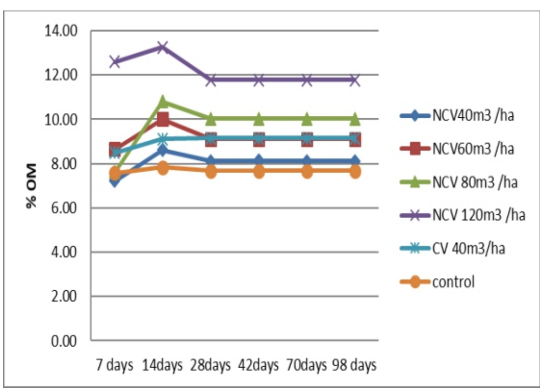

Figure 3: Variation of OM values with different levels of NCV and CV with the time in laboratory condition

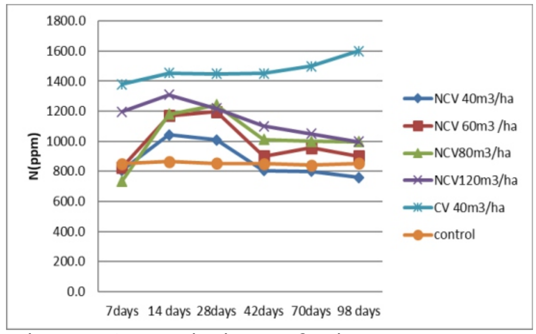

Figure 4: Variation of nitrogen content values in different levels of NCV and CV with the time in laboratory condition

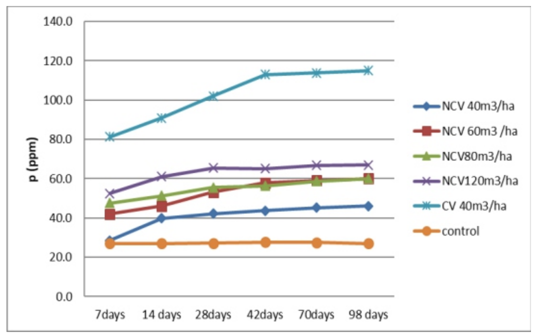

Figure 5: Variation of phosphorus content values in different levels of NCV and CV with the time in laboratory condition

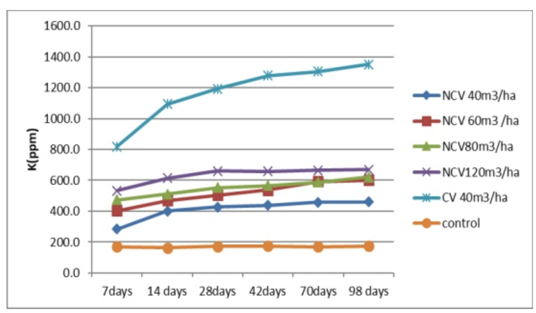

Figure 6: Variation of potassium content values in different levels of NCV and CV with the time in laboratory condition

Effects of sugarcane vinasse on initial growth of sugarcane varieties SL 83 06 and SL 96 128

Figures 7 and 8 present the effect of different levels of vinasse on improvement in shoot length (SL), root length (RL), root dry weight (RDW), shoot dry weight (SDW) of sugarcane variety SL 8306 and SL 96 128. Calculated index values of each growth attribute were compared and results revealed that very low rates of application of NCV (40 m^3/ha) showed better improvement in measured parameters for both varieties. However, variety SL 83 06 showed better performances than SL 96 128 for almost all tested growth attributes.

The variety SL 83 06 showed more or less similar performance as SL 96 128. The Highest improvement of Growth attributes was found in the 40 m^3/ha NCV level, closely followed by the 60 m^3/ha NCV level. SL 83 06 showed improvement in 80% in SDW, 90% in RL, 58 % in SDW, and 74 % in RDW in 40m^3/ha NCV level.

The variety SL 96 128 also showed the best performance in 40 m^3/ha NCV level, followed by 60 m^3/ha for measured growth parameters which was 80% improvement in SDW 38% in SL, 92 % in RDW, and 32 % in RL. Overall, plant performance decreased with the increasing rate of vinasse application in the pot experiment.

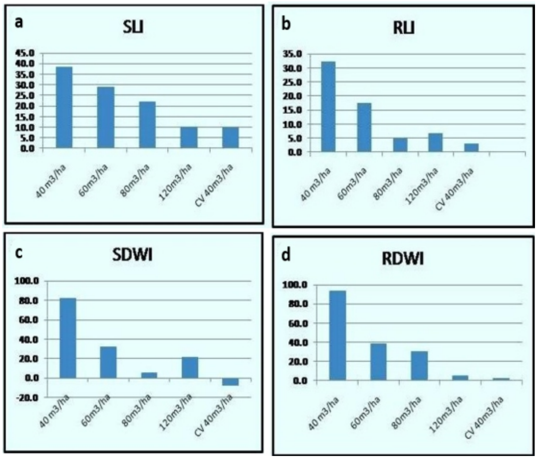

Figure 7: Effect of vinasse on bud sprouting (a), shoot length (b) root length (c), shoot dry weight (d), root dry weight of sugarcane variety SL 83 06

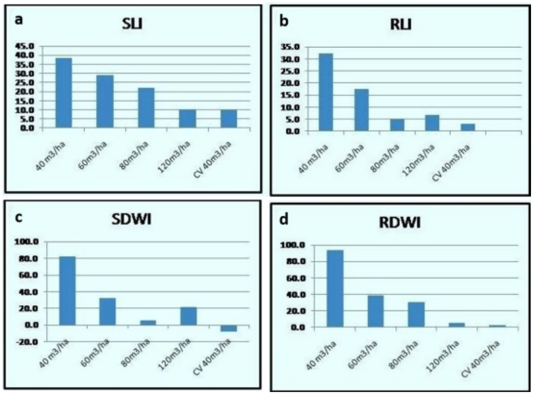

Figure 8: Effect of vinasse on bud sprouting (a), shoot length (b) root length (c), shoot dry weight (d), root dry weight of sugarcane variety SL 96 128

CONCLUSION

According to the results of the experiment, it could be concluded that the direct soil application of vinasse is a feasible method for its disposal. Also, it had a direct effect as a good source of elements and an indirect effect consisting of improvement of initial growth attributes of SL 96128 and SL 8306. Finally, results revealed that the lower doses of non-concentrated vinasse are more favorable to plant growth with a stimulatory effect on plant initial growth.

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