Acta Iguazu

ISSN: 2316-4093

Validation of leaf cover analysis software (LCAS) to monitor lettuce cultivated with organic fertiliser

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Artigo enviado em 11/11/2017, aceito em 20/11/2018.

Abstract: Composting and vermicomposting are techniques used to stabilise organic waste and produce nutrient-rich fertilisers. Experiments that analyse cultures and different fertilisers applied to soil require a heavy workload and time to monitor plant development. Thus, alternative methods to facilitate this monitoring need to be created. The aim of this paper was to assess the development of lettuce cultivated with eight types of fertilisers derived from composting and vermicomposting, and to compare the traditional data collection method with leaf cover analysis software ("LCAS"). The assessed items were growth, diameter, number of leaves, fresh mass, dry mass, and leaf cover obtained using the software. Overall, the lettuce cultivars that received organic fertiliser developed better than the witness, especially in relation to treatments containing ash, such as the C4 compost (Mud, Ash and Pruning litter) and V2 vermicompost (Mud, Ash and Coffee husk). LCAS proved to be an effective tool to monitor the growth of lettuce cultivars in comparison with the traditional method since statistically the pattern of behaviour of the treatments was similar for plant cover, diameter, length, and fresh and dry masses.

Key Words: composting, *Lactuca sativa L*, solid waste.

Validação de software de análise de cobertura foliar (LCAS) para monitorar alface cultivada com adubo orgânico

Resumo: Compostagem e vermicompostagem são técnicas usadas para estabilizar resíduos orgânicos e produzir fertilizantes ricos em nutrientes. Experimentos que analisam culturas e diferentes fertilizantes aplicados ao solo requerem uma carga de trabalho pesada e tempo para monitorar o desenvolvimento da planta. Assim, métodos alternativos para facilitar esse monitoramento precisam ser criados. O objetivo deste trabalho foi avaliar o desenvolvimento de alface cultivada com oito tipos de fertilizantes derivados de compostagem e vermicompostagem e comparar o método tradicional de coleta de dados com o software de análise de cobertura foliar ("LCAS"). Os itens avaliados foram: crescimento, diâmetro, número de folhas, massa fresca, massa seca e cobertura foliar obtida por meio do software. No geral, as cultivares de alface que receberam adubação orgânica apresentaram melhor desenvolvimento que a testemunha, principalmente em relação aos tratamentos contendo cinzas, como o composto C4 (Lodo, Cinza e Poda) e V2 vermicomposto (Barro, Cinza e Café). O LCAS mostrou-se uma ferramenta eficaz para monitorar o crescimento de cultivares de alface em comparação ao método tradicional, uma vez que estatisticamente o padrão de comportamento dos tratamentos foi semelhante para cobertura vegetal, diâmetro, comprimento e massas frescas e secas.

Palavras-chave: compostagem, *Lactuca sativa L*, resíduos sólidos.

Introduction

The dairy industry is a sector that characteristically consumes high volumes of water during the production process and, consequently produces large volumes of wastewater from the cleaning of tanks, silos, homogenizers and other equipment. This wastewater has a high organic load, and high levels of oils, fats, nitrogen, phosphorous, and chemical products (SINGH et al., 2014). The most commonly used treatment is biological, which results in a white sludge with an unpleasant odour, a high percentage of humidity, and high levels of organic matter (SUTHAR et al., 2012).

There are two processes to stabilise organic matter that can also be used to treat sludge: composting, which is entirely the work of micro-organisms; and vermicomposting, which is when earthworms form symbiotic relationships with the microorganisms that live in their digestive tract (DORES-SILVA et al., 2013). The result of this process is a fertiliser that can be applied to the soil for agricultural purposes to recover the physical, chemical, and biological properties of soil and increase crop production (MURWONO et al., 2011).

The application of these fertilisers to soil should be monitored, especially for crop cultivation. In practice, conventional monitoring (height, diameter, and fresh and dry mass) are time consuming and require a lot of manpower. Digital imagery can be used to efficiently monitor plant growth (FERNÁNDEZ-PACHECO et al., 2014) and assess canopy cover at a lower cost. Image analysis provides important quality parameters such as colour, which helps to effectively assess canopy cover and plant maturity (CAMELO-MÉNDEZ et al., 2012). In addition, according to De Jesus et al. (2001) and Michels et al. (2018), vegetation indexes are essential to study plant growth and better understand plant-environment interactions.

Therefore, the aim of this paper was to verify the correlation between the traditional method of monitoring (fresh and dry mass, diameter, growth, and number of leaves) and Leaf Cover Analysis Software (LCAS) in the development of lettuce cultivated with different organic fertilisers.

Materials and Methods

The organic fertilisers were obtained by means of composting and vermicomposting processed in covered and uncovered settings. We used different agro-industrial waste, totalling four composts, four vermicomposts and one witness that did not receive fertiliser (Table 1).

Table 1. Treatments applied to lettuce cultivars according to the compost and vermicompost processing settings

Setting	Composting
Covered	C1 - Sludge and Coffee husk C2 - Sludge, Ash and Coffee husk
Uncovered	C3 - Sludge and Pruning litter C4 - Sludge, Ash and Pruning litter
Setting	**Vermicomposting**
Covered	V1 - Sludge and Coffee husk V2 - Sludge, Ash and Coffee husk
Uncovered	V3 - Sludge and Pruning litter V4 - Sludge, Ash and Pruning litter

The wastes used to obtain these fertilisers were sludge from the wastewater treatment plant through an activated sludge system in the dairy factory, tree pruning, boiler ash, and coffee husks. The physical and chemical properties of the fertilisers are shown in Table 2.

Table 2. Physical and chemical characteristics of compost and vermicompost

Treatment	pH	EC (μS cm^{-1})	N (%)	C (%)	C/N
C1	8,49	2210	2,94	31,08	10,56
C2	8,81	1915	1,68	29,84	17,78
C3	6,14	2178	2,62	38,00	14,51
C4	7,71	628	1,60	30,76	19,23
V1	7,93	2298	2,75	34,35	12,51
V2	7,66	2960	2,27	23,49	10,35
V3	6,58	966	2,98	33,21	11,16
V4	7,61	342	1,49	23,76	15,91

Note 1: pH - Power of Hydrogen; EC - Electrical Conductivity; Nitrogen (N) and Carbon (C) – Levels of nitrogen and carbon in the compost and vermicompost; C/N - Carbon/Nitrogen ratio.

In the composting process, the plots were assembled in a trapezoidal format, each with an initial volume of 200 L. The process lasted 57 days, during with the thermophilic and mesophilic stages were observed. In the subsequent vermicomposting period, 24 worms of the species *Eisenia foetida* were placed in reactors with a volume of 25 L each, for 43 days.

Lettuce cultivation

The lettuce was cultivated in the facilities of the Universidade Tecnológica Federal do Paraná, Londrina Campus, located at 23°18'32.1" S latitude, 51°07'00.1" W longitude, and at an average altitude of 610 metres above sea level.

The soil in the region is predominately distropheric Red Latosol (TRABAQUINI et al., 2010). Table 3 shows the chemical characteristics of the soil, based on the methodology of EMBRAPA.

Table 3. Chemical characteristics of the soil (0-20 cm depth) of the cultivation area

pH	P	C	Al	H + Al	Ca	Mg	K	SB [1]	T [2]	V [3]	SAI [4]
	mg dm^{-3}	g dm^{-3}	———————			$cmol_C$ dm^{-3}		———————		%	
5,6	11	8,18	0	3,97	6,5	1,48	0,27	8,25	12,22	67,51	0

Note 1: SB = sum of bases
Note 2: CEC = cation exchange capacity
Note 3: Base saturation
Note 4: Aluminium saturation.

The cultivation of lettuce observed the methodology indicated by EMBRAPA (2006), with some adjustments. A shade cloth structure was built to block any direct sunlight on the lettuce. The plants were produced in beds in the soil, as indicated for leafy green vegetables. Two beds were prepared with a height of 0.11 m, a base width of 1.40 m, a crest width of 1.20 m, a crest length of 3.90 m, and a base length of 4.10 m. The spacing between each plant was 0.30 m, recommended by Lúcio et al. (2011), and the useful planting area in each bed was 4.68 m^2.

The chosen lettuce (*Lactuca Sativa* L.) was the cultivar Vera because it is safe to plant in the summer and adapts well to tropical conditions.

Each treatment had a useful plot with six repetitions. In addition to these plots, there was a ridge row surrounding each bed and between each treatment to prevent any wind or sunlight from favouring the useful plot.

The treatments along the crop area were distributed randomly, by means of draws.

According to EMATER (2007), the amount of nitrogen required for lettuce to grow correctly is 80 kg ha^{-1} (8 g m^{-2}). By knowing the required amount of nitrogen for lettuce growth and the amount of this nutrient in the compost, it was possible to calculate the amount of fertiliser in the dry matter base that was added to each useful planting area (Table 4).

Table 4. Amount of fertiliser per plot for each treatment, value calculated according to the nitrogen nutrition needs of the lettuce cultivars (80 kg ha^{-1})

Treatments[1]	N (g kg^{-1})	Amount of fertiliser (g)[2]
C1	29,43	271,87
C2	16,78	476,72
C3	26,19	305,46
C4	16,00	500,01
V1	27,46	291,34
V2	22,71	352,31
V3	29,76	268,83
V4	14,93	535,69

Note 1: C1 - Sludge and Coffee husks; C2 – Sludge, Ash and Coffee husks; C3 - Sludge and Tree prune litter; C4 - Sludge, Ash and Tree prune litter; V1 - Sludge and Coffee husks; V2 - Sludge, Ash and Coffee husks; V3 - Sludge and Tree prune litter; V4 - Sludge, Ash and Tree prune litter
Note 2: Calculated in dry matter basis. This amount was applied to the entire plot area (1.17 m^2).

Cultivar development assessment

Lettuce development was assessed according to the parameters fresh mass (FM), dry mass (DM), average head diameter (HD), average leaf growth (LG), number of leaves (NF), and leaf canopy using the LCAS.

Only the aerial part of the plants was used to determine fresh mass (FM) and dry mass (DM), as recommended by Lúcio et al. (2011). At the end of the 40-day planting period, the lettuce cultivars were harvested and the aerial part was separated from the stem. The lettuce plants were weighed on an analytical scale to obtain FM. After weighing the plants were placed in paper bags and identified, and these samples were placed in a forced-air oven at 65° C for 72 hours (VIDIGAL et al., 1997). After this period, the samples were weighed to determine the DM of each plant.

The diameter of the heads (HD), average leaf growth (LG), and number of leaves (NL) were evaluated in the field on the 40th day of cultivation. A ruler was used to obtain HD. The LG was calculated by measuring the neck of the plant until the apex of the last leaf (ALMEIDA et al., 2011).

Leaf cover assessment

The images were obtained using a digital camera. To ensure data standardisation, a support of 90 cm in height was built to take the pictures.

The images were obtained from the top to include the 6 lettuce cultivars (Figure 1) of each plot in the frame. Five photos were taken every day after the 14th day of planting, always at the same time (7:30 a.m.) to ensure the same brightness in the pictures.

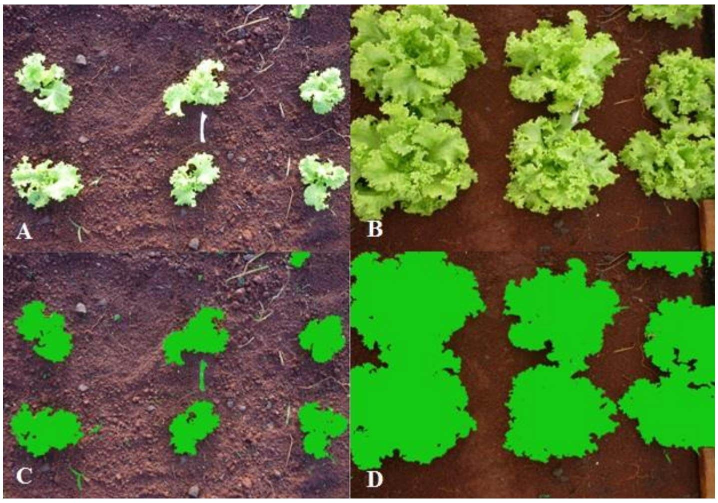


Figure 1. Digital images pre- (A-B) and post- (C-D) treatment in the LCAS

The images were subsequently analysed using LCAS, which provides plant cover (%) of the image by analysing the total percentage of green pixels.

Statistical data analysis

The results of leaf cover, diameter, length, and fresh and dry masses were statistically analysed to detect any significant difference between the treatments and between the development analysis methods.

Analysis of variance was performed at a 5% level of significance and the Scott-Knott test was used to compare averages.

The Pearson correlation coefficient was obtained, followed by regression analysis between leaf cover and the other assessed parameters (LG, HD, FM, DM, and NL).

Results and Discussion

The soil containing organic matter from compost and vermicompost significantly improved the development of the lettuce cultivars in comparison to the witness. Table 5 shows this comparison and the statistical difference at 5% significance in the Scott Knott test between the witness (T0) and the other treatments.

Table 5. Comparison of averages according to the Scott Knott statistical test of head diameter (HD), leaf growth (LG), dry mass (DM), fresh mass (FM), number of leaves (NL), and leaf cover between treatments and Pearson's correlation coefficient between the Leaf Cover Method using Leaf Cover Analysis Software (LCAS) and the traditional methods

Tratamento	LCAS Method	Traditional Method				
	Leaf Cover (%)	DC (cm)	CF (cm)	MS (g)	MF (g)	NF
T0	3,650 A	10,333 A	6,833 A	0,045 A	6,025 A	7,167 A
V1	32,220 B	20,167 B	12,000 B	2,760 B	44,828 B	12,400 A
C2	32,996 C	19,667 B	11,416 B	1,613 A	32,043 B	10,833 A
V3	33,386 C	19,333 B	12,333 B	2,325 B	38,341 B	12,500 A
V4	35,594 D	22,500 C	13,167 B	2,826 B	38,778 B	14,167 B
C3	38,870 E	19,500 B	12,000 B	1,638 A	39,876 B	15,333 B
C1	47,172 F	23,000 C	14,167 C	2,995 B	54,265 C	14,500 B
V2	50,488 G	23,833 C	14,833 C	4,075 B	65,878 C	16,667 B
C4	54,482 H	26,000 C	15,167 C	4,563 B	82,923 C	17,667 B
Pearson's Correlation	--	0,967	0,98	0,893	0,939	0,940

Note 1: The same letters in the columns imply statistical equivalence of the values at a 5% level of significance.
Note 2: (T0), Sludge and Coffee husks (C1); Sludge, Ash and Coffee husks (C2); Sludge and Tree prune litter (C3); Sludge, Ash and Tree prune litter (C4); Sludge and Coffee husks (V1); Sludge, Ash and Coffee husks (V2); Sludge and Tree prune litter (V3); Sludge, Ash and Tree prune litter (V4).

The diameter of the lettuce heads of treatments C1, C4, V2 and V4 was statistically greater at a 5% level of significance compared to the other treatments, and the witness (T0) got the worst result.

Regarding the length of the leaves, the treatments with the best results at a 5% level of significance were C1, C4 and V2, and the witness got the worst result. Gonçalves et al. (2014) planted lettuce with organic compound from plant debris, boiler ash, and sawdust, and also found a greater average length of the lettuce planted with organic fertiliser in comparison to the lettuce grown without this compost.

Fagnano et al. (2011) found that lettuce cultivated with the organic compounds from the composting of the organic fraction of urban waste had positive effects on soil fertilisation, and claims that the doses of organic compounds must be balanced to reduce the risk of nitrate leaching during the rainy season. Consequently, the successful development of lettuce seedlings in soil with organic compound or vermicompound suggests that this substrate favours this species.

A comparison of the Scott Knott average at 5% significance of the fresh mass showed that treatments C1, C4 and V2 got the best results, while in the dry mass the witness (T0) and treatments C2 and C3 got the worst results. These results partially resembled the parameter number of leaves, where the worst results were found in treatments C2, V1 and V3, and in the witness (T0).

The best results of leaf cover analysis using LCAS were obtained for treatments C1, C4 and, V2, which matches the results obtained for the parameters HD, LG, FM, and NL.

It was also observed that treatments C4 and V2 had the greatest leaf cover at a 5% level of significance. Both treatments contained ashes, which could have favoured the development of the lettuce cultivars grown in these compounds. Juárez et al. (2015) added ash to compost made from garden waste, food, paper and compostable packaging fibres, and noticed an increase in the nutrient content of the compost.

Pearson's correlation between leaf cover obtained using LCAS and the parameters HD, KG, FM, and NL showed values higher than 93.9%. This high correlation indicates that the parameters are intrinsically linked, and that LCAS is a good alternative to analyse the development of crops since it enables data collection and highly correlated with manually obtained data.

Figure 2 shows the leaf cover behaviour from the 14th day after planting until harvesting for the treatments with compost (A) and the treatments with vermicompost (B).

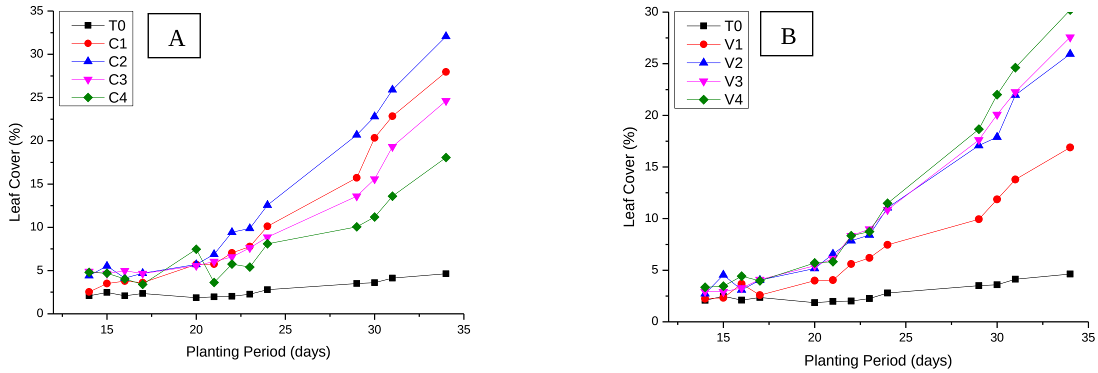
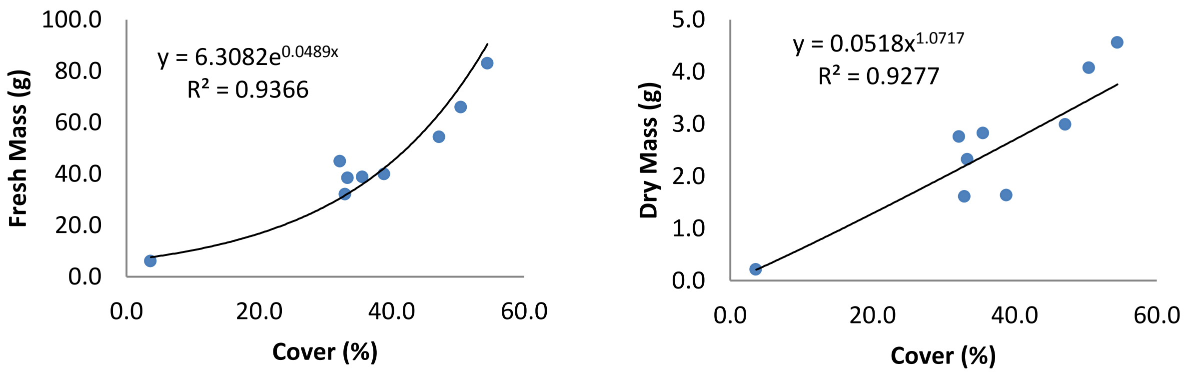


Figure 2. Leaf cover behaviour during lettuce cultivation for treatments with compost (A) and treatments with vermicompost (B).

As shown in Figure 2, the lettuce grown with compost and vermicompost developed better during the study period than the lettuce that was planted with soil only (T0). Castoldi (2014) assessed the efficiency of alternative substrates for planting lettuce seedlings and observed better results among the plants grown with worm compost due to the higher levels of nutrients this compost provides.



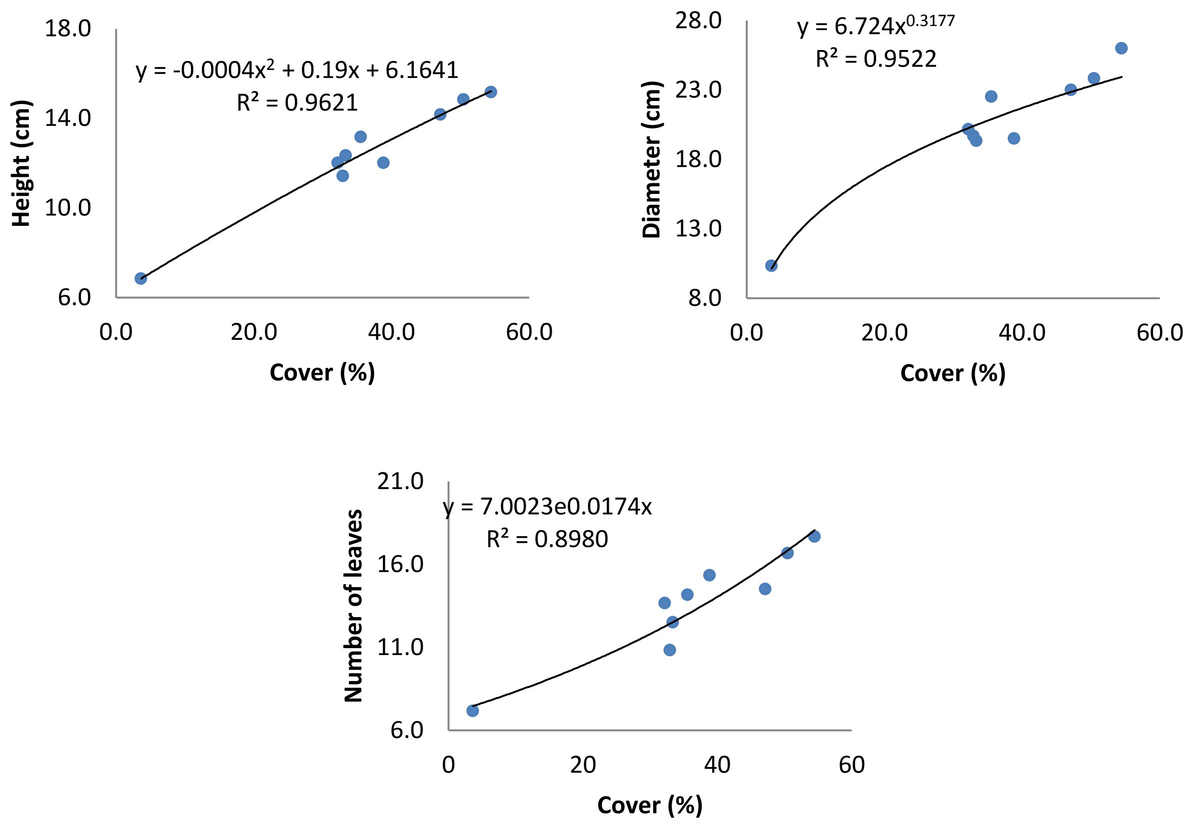


Figure 3. Regression between LCAS and parameters head diameter, length of the leaves, fresh mass, dry mass, and plant height.

The regressions shown in Figure 3 confirm the use of LCAS to monitor the performance of lettuce in experiments.

Figure 3 shows that the correlation between the LCAS and the traditional monitored parameters is at least 89.8%, which reinforces the possibility of using LCAS to monitor the development of lettuce cultivars.

Baumgarner et al. (2012) compared the use of digital imaging and the traditional method of analysis to assess the biomass growth of lettuce, and found that digital imaging and the subsequent treatment in analysing software reduces the amount of samples and certain tools that can delay the collection of some data.

Conclusion

Results showed that the development of lettuce grown with organic fertiliser was better than the development of lettuce grown only with soil. This finding indicates that organic fertilisers can be used to replace generally commercialised substrates, and as an alternative to reuse organic solid waste generated in certain types of agro industries.

Treatments C4 and V2 got the best results in the leaf cover analysis.

LCAS proved to be a viable alternative when compared to the traditional methods of monitoring lettuce canopy development.

Acknowledgements

The authors would like to thank the UTFPR and the Araucaria Foundation for the grant that funded this research.

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