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Research article

EFFECT OF *EUCALYPTUS CAMALDULENSIS* LEAF AQUEOUS EXTRACT ON MORPHOLOGY, PHYSIOLOGY AND BIOMASS OF *ZEA MAYS*

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ABSTRACT

The seeds of *Zea mays* were disinfected by 5% sodium hypochlorite. In a pot experiment the aqueous leaf extract of *E. camaldulensis* was applied in three different concentrations along a control. Results revealed that height, girth diameter, and leaf area, chlorophyll florescence, total soluble sugar, protein content, nitrogen contents, shoot dry weight (g), and relative biomass ratio showed significant variation whereas number of green leaves and root dry weigh (g) had not showed variation at P≤0.05. Among the concentrations 30% aqueous leaf extract showed high negative impact than 10% and 20% extracts. Further, the analysis revealed biomass had positive correlation with almost all traits and have been found to be effective screening tool for allelopathic stress.

Key Words: Allelopathy, Aqueous extract, *Eucalyptus camaldulensis*, Zea mays.

INTRODUCTION

Eucalyptus is non-edible plant which occupies farm land intended for food crops cultivation and may have negative effect on indigenous plant species by competing resources (light, water and soil nutrient) (Perez *et al.,* 2001). It also, produces allelopathic compounds. Allelophathy is a biological phenomenon by which an organism produces inhibitory biochemicals that limits the growth, physiology, biochemical, biomass, and reproduction of other organisms (Alireza and Ali, 2010). It impairs normal growth (Selamyihun *et al*., 2005), disturbs water status and chlorophyll fluorescence (Gareca *et al.,* 2007), malfunctions the photosynthesis path way (Alireza and Ali, 2010), increase accumulation of osmolytes (inorganic ions, sugars and amino acids) within the cell (Gareca *et al.,* 2007) and affects the yield of various crops (Onyewotu *et al.,* 1994; Dawar *et al*., 2007).

Several studies were conducted in the Ethiopian highland areas and the findings showed that the reduction in the growth, physiology, biochemical, biomass and yield of different crops when grown

close to eucalypts (Selamyihun *et al*., 2005; Tilashwork *et al*., 2013). Eucalypts have been reported to cause crop loss by outcompeting crops for water and soil nutrients, through shading and producing allelochemicals or allelopathy (Amare, 2002; Dereje *et al*., 2012; Tilashwork *et al*., 2013). On the other hand, the crop maize is an important crop which is highly affected by aqueous leaf extract of *E. camaldulensis* (Siddiqui and Zaman, 2004; Jiregna, 2006).

MATERIAL AND METHODS

Aqueous leaf extraction

The fresh leaves of *E. camaldulensis* were collected from its mono culture stand growing at Tewodros campus, University of Gondar, Ethiopia. The fresh leaves were dried at room temperature and grinded by electrical grinder. The powder was soaked with water in the ratio of 1:20 (w/v) and kept for 72 h and the filtrate was designated as stock solution. From the stock solution other concentrations (10%, 20% and 30%) were prepared with water (Hussain and Gadoon, 1981). 100 ml of each (treatment and control) was applied per day.

Experimental design and pot management

The pot experiment was performed using random complete block design (RCBD) with three different concentrations (10%, 20% and 30% extracts) and one control group (water) with three replications (3*4). The seeds were sowed at 35cm diameter pots, filled with growth media. The media were prepared by mixing sand, soil and compost with the ratio of 1:2:1. Its pH and the texture were determined using modified Juo (1978) method. While the electric conductivity of the soil was estimated using calibrated conductive meter. The pH, texture and electric conductivity were 7.5, silt clay loam and 970µS cm^{-1}. Five seeds of maize were sowed per pot and the recommended rate of fertilizer was applied within the rate of 100/50 kg per hectare Urea [$CO(NH_2)_2$], and DAP [$(NH_4)2$ HPO4]. Weeding and all other recommended crop protection activities were done manually, recommended for maize cultivation. Growth, physiological, and biochemical traits were measured at 60 days after sowing while biomass were determine at 95 days after sowing.

Morphological and physiological traits

Plant height, girth diameter, number of green leaves, and leaf area were determined. Number of green leaves (more than 50% green portion) was counted and the leaf area was measured by using leaf area meter (AM 300, ADC Bio SC Limited, England). In addition the relative elongation of shoot was determined based on Rho and Kil (1986) method using the following formula.

$$\text{RERs} = \frac{MLSt}{MLSc} x100\%$$

Where, RERs is the Relative elongation Ratio of shoot, MLSt is the Mean Length of Shoot of plant under treatments and MLSc is the Mean Length of Shoot of plant under control. The chlorophyll fluorescence (Fv/Fm) was measured in the forenoon (10:00 to 11:00 am) using portable Multi-Mode Chlorophyll Fluorometer (ADC Bio Scientific Ltd., England). Fv/Fm was determined from the youngest and fully expanded leaves of maize. The leaves were dark adapted by covering it with the clip and kept in dark for half an hour before measurements.

Biochemical traits

Chlorophyll contents were estimated according to the method of Hiscox and Israelstam (1979). Leaf total soluble sugar was estimated by using phenol sulphuric acid method (DuBois *et al*., 1956) and the leaf nitrogen content was estimated by modified micro Kjeldhal method (Jackson, 1973) with three steps such as digestion, distillation and titration.

Biomass determination

The shoot and root parts of each treatment was separated and dried in the oven at 80^0 C until constant weight was obtained. Consequently, the dry weights were determined using a balance. Relative biomass ratio was measured based on the formula modified by Rho and Kil (1986).

$$\text{RBR} = \frac{MBt}{MBc} X100$$

Where RBR is the Relative Biomass Ratio, MBt is the Mean Biomass of plant under treatment and MBc is the Mean Biomass of plant under control.

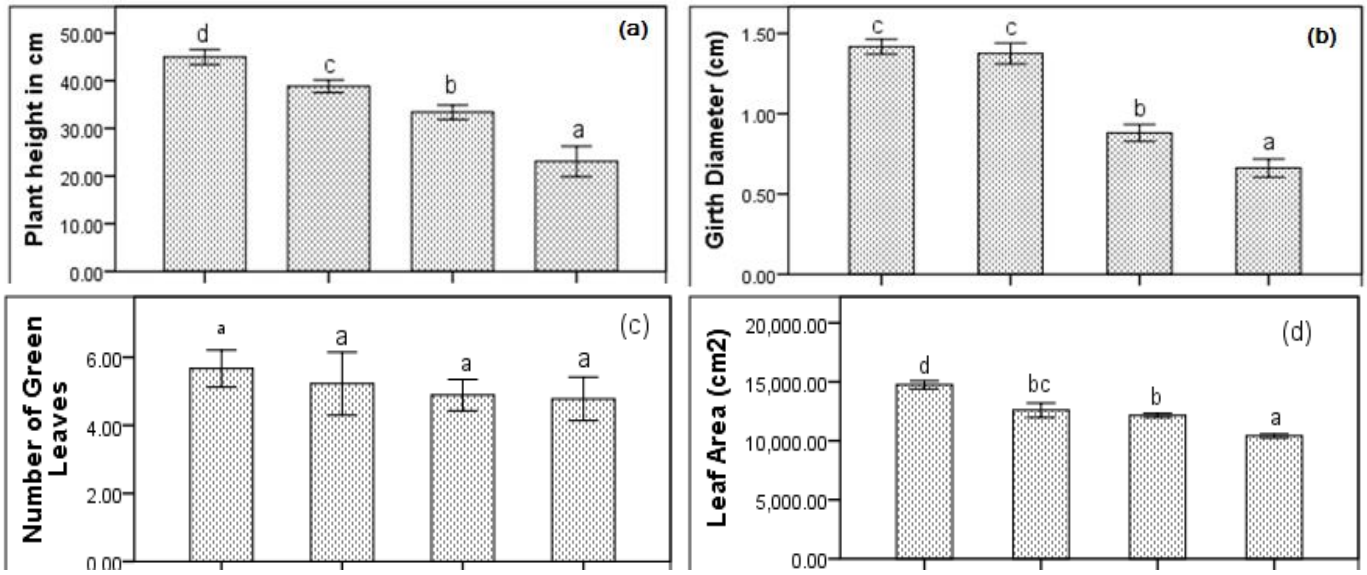
Statistical analysis

All the collected data were subjected to analysis of variance using SPSS software (Version 20, SPSS Inc., Chicago USA). The difference in concentration mean were separated using Leas significant difference (LSD) test at 5% level of significant. The correlations between the studied traits were analyzed using Pearson's test.

RESULT

Morphological and physiological traits

Data showing the effect of *E. camaldulensis* aqueous leaf extract on the studied plant growth (morphology) and physiology are presented in Figure.1. The present study revealed that, control followed by 10% aqueous leaf extract showed significantly maximum plant height, while the minimum was recorded for 30% aqueous leaf extract (Figure.1a). The maximum girth diameter value was observed with control, which exhibited insignificant variation with 10% aqueous leaf extract and the lowest was recorded with 30% aqueous leaf extract (Figure. 1b). Number of green leaf formation has not significantly affected by *E. camaldulensis* and among the concentrations significant variation were not observed Figure 1c).



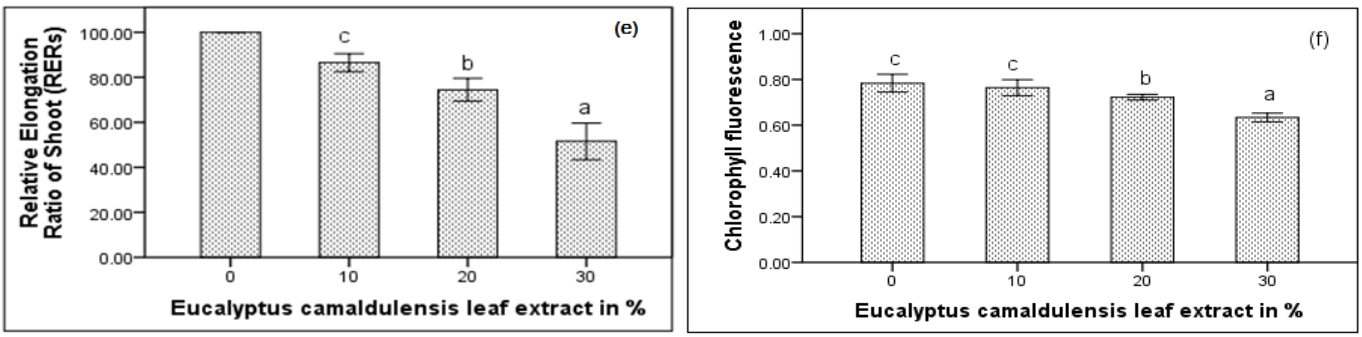


Figure 1. Effect of *E. camaldulensis* aqueous leaf extract on morphological and physiological traits. (a) Plant height, (b) Girth Diameter, (c) Number of green leaves, (d) Leaf area, (e) Relative Elongation Ratio of Shoot (RESs), (f) Chlorophyll fluorescence of maize at 60 days after sowing. Bars represent mean (± SE) of three replicates and designated with the same letter are not significantly different at p≤0.05.

The leaf area exhibited a reduction trend when the concentration increase, The mean value varies from 10416mm^2-14753 mm^2, the maximum was observed at control followed by 10% aqueous leaf extract while the minimum was 30% aqueous leaf extract (Figure 1d). The highest relative elongation ratio of shoot was found more in 10% aqueous leaf extract which exhibit significant variation with 20% and 10% aqueous leaf extract (Figure 1e). The mean value of quantum yield of PSII efficiency (Fv/Fm) showed inverse relation with the concentration of the leaf extract. The maximum quantum value was found among the control (0.78) and 10% aqueous leaf extract, while the least value was at 30% aqueous leaf extract. Among concentrations 10% aqueous leaf extract was not significantly varied with control group, while others were significantly varied (Figure 1f).

Biochemical traits

Photosynthetic pigments, nitrogen, protein, and soluble sugar contents, were estimated and the result is given in Table 1. Chlorophyll a, chlorophyll b and total chlorophyll content showed significant variation and found more in the control and 10% aqueous leaf extract while the least was recorded at 30% aqueous leaf extract. Among the concentration, control and 10% aqueous leaf extract were insignificantly varied while others showed significant variation (Table 1). Total soluble sugar, leaf nitrogen and protein content were highest in 10% aqueous leaf extract, while 30% aqueous leaf extract exhibited the lowest value. Among the groups, 20% aqueous leaf extract and 30% aqueous leaf extract variation were not significant while other groups were significant in total soluble sugar, leaf nitrogen and protein contents (Table1).

Table 1. Biochemical activities of maize as measured on 60 days of sowing.

S. No	Parameters	(Distilled water) 10%	10%	20%	30%
1	Chlorophyll a (mg LG^1 f. wt.)	0.77 ± 0.02^c	0.75 ± 0.01^c	0.67 ± 0.03^b	0.36 ± 0.06^a
2	Chlorophyll b (mg LG^1 f. wt.)	0.80 ± 0.03^c	0.78 ± 0.07^c	0.72 ± 0.04^b	0.42 ± 0.05^a
3	Total chlorophyll (mg LG^1 f. wt.)	2.70 ± 0.08^c	1.72 ± 0.13^c	1.60 ± 0.31^b	1.11 ± 0.51^a
4	Soluble sugar (µg mLG^1 D. wt.)	9.16 ± 0.20^b	9.28 ± 0.37^b	6.73 ± 0.23^a	6.59 ± 0.25^a
5	Nitrogen (%)	5.61 ± 0.23^c	4.58 ± 0.25^b	3.02 ± 0.12^a	2.99 ± 0.55^a
6	Protein (%)	30.28 ± 0.30^c	27.93 ± 0.43^b	15.14 ± 0.68^a	14.57 ± 0.35^a

Data represents mean ±SE of the three replicates. Means followed by the same letter in a row are not significantly different at $p\leq0.05$.

Biomass traits

The shoot dry weight (SDW) and root dry weight (RDW) were estimated on and given in Figure 2. The Shoot Dry Weight (SDW) showed significant variation. Among the groups all concentrations of SDW differ significantly and 30% aqueous leaf extract have exhibited significantly lower SDW (Figure 2a). Insignificant variation was found in Root Dry Weight (RDW) (Figure 2b). Relative biomass (relative to the control) also showed significant variation (Figure 2c). The maximum value was recorded on the 10 % extract (89.5) followed by 20 % extract (86.9) whereas the minimum relative biomass was recorded at 30 % leaf extract (68.8) (Table 2). Among the groups each concentration mean value were significantly differ each other (Figure 2c).

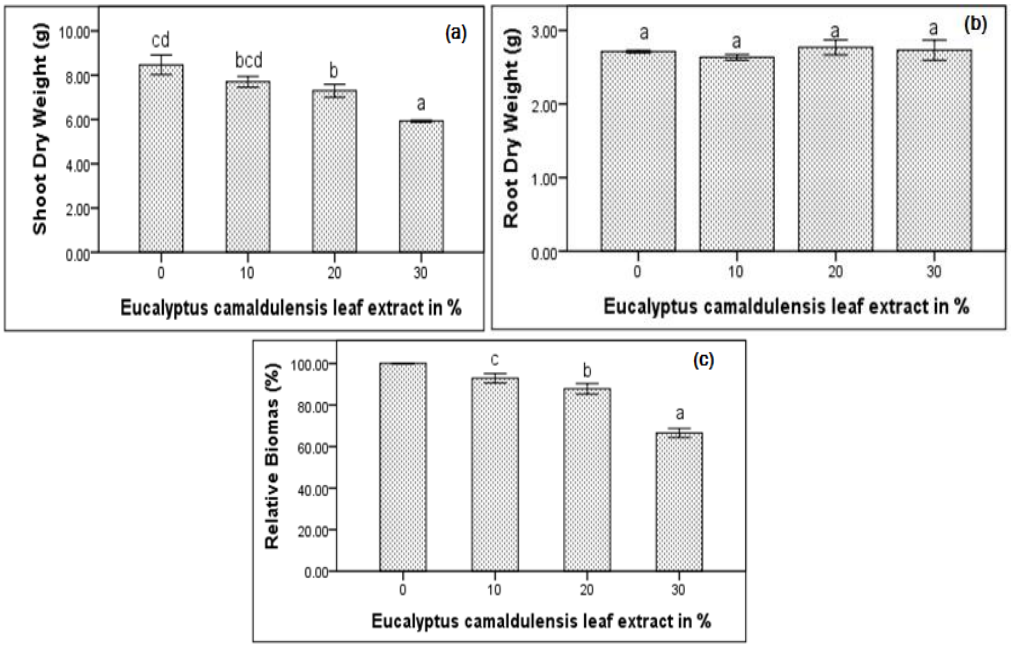


Figure 2. Shoot (a), Root (b) and relative (c) biomass of maize at 95 days after sowing. Bars represent mean ±SE of the three replicates and designate with the same letter are not significantly different concentration at $p\leq0.05$.

Correlation studies

Correlation was performed for various parameters and presented in Table 2. Plant height was positively correlated with leaf area, chlorophyll content, shoot dry weight, root dry weight, and RERs but not with number of green leaves under this experiment. Leaf area had a strong positive correlation with all traits except number of green leaf and root dry weight. Biomass had positive correlation with plant height, leaf area, Fv/Fm, relative biomass, and chlorophyll contents, soluble sugar, nitrogen and protein contents. But root dry weight had no correlation with leaf area, Fv/Fm, sugar and protein contents. Chlorophyll surprisingly strongly correlated with all traits under this field trial. Total soluble sugar, nitrogen and protein content had showed strongly positive correlation with all traits except root dry weight.

Table 2. Pearson's correlation study among the different morphological, physiological, biochemical and biomass traits of maize.

Traits	Height	Leaf Area	Fv/Fm	GD	NGLs	SDW	RDW	RB	RERs	Chl a	Chl b	T chl	Sugar	LN	Protein
Height	1	0.952**	0.950**	0.865**	0.283	0.888**	0.505**	0.766**	0.981**	0.905**	0.891**	0.856**	0.819**	0.853**	0.850**
Leaf Area		1	0.905**	0.843**	0.437	0.931**	0.528	0.749**	0.950**	0.859**	0.843**	0.960**	0.770**	0.897**	0.833**
Fv/Fm			1	0.928**	0.470	0.921**	0.571	0.798**	0.939**	0.975**	0.960**	0.792**	0.829**	0.803**	0.841**
GD				1	0.421*	0.840**	0.422*	0.729**	0.847**	0.863**	0.831**	0.765**	0.949**	0.897**	0.946**
NGLs					1	0.270	0.028	0.211	0.273	0.299	0.263	0.362*	0.351*	0.383*	0.340**
SDW						1	0.656**	0.921**	0.880**	0.898**	0.898**	0.838**	0.752**	0.804**	0.805**
RDW							1	0.750**	0.502**	0.631**	0.652**	0.449**	0.289	0.332*	0.323
RB								1	0.742**	0.786**	0.800**	0.640*	0.587*	0.610*	0.650**
RERs									1	0.890**	0.880**	0.839**	0.810**	0.840**	0.839**
Chl a										1	0.995**	0.723**	0.760**	0.706**	0.760**
Chl b											1	0.711**	0.718**	0.668**	0.720**
T chl												1	0.697**	0.899**	0.774**
Sugar													1	0.917**	0.986**
LN														1	0.956**
Protein															1

Fv/Fm: Chlorophyll fluorescence, GD: Girth diameter, NGLs: Number of Green leaves, SDW: Shoot Dry Weight, RDW: Root Dry Weight, RB: Relative Biomass, RERs: Relative Elongation Ratio of shoot, Chl a: Chlorophyll a, Chl b., chlorophyll b, T Chl: Total Chlorophyll, N: Leaf nitrogen. ** and *: Significant at $p \leq 0.01$ and $p \leq 0.05$ levels (n = 60-90).

DISCUSSION

Leaf aqueous extracts of *E. camaldulensis* exerted an increased negative impact on the growth, physiology, biochemicals, and biomass of maize towards increased concentration. It was found that plant height, girth diameter, leaf area, relative elongation ratio of shoot, and chlorophyll florescence were more with 10% aqueous leaf extract next to the control. Plant height variation could be due to the extract containing some phytotoxic substance that contains inhibitory effect. Similarly, the shoot growth of some herbaceous plant like chick pea, maize, pea and teff are inhibited due to an aqueous leaf extract of *E. camaldulensis* (Guo *et al.*, 2006; Desalegn and Demel, 2014).

Our study correlated with Suresh and Rai (1987) studies, which showed a strong

reduction of shoot growth, root height and dry matter production in sorghum, cowpea and sunflower in cultivating with eucalyptus. Girth diameter significantly affected by eucalyptus aqueous leaf extract in the present study is in agreement with Selamyihun *et al*. (2005) who found in wheat but not in other crops like Sorghum (Hussain and Gadoon, 1981; Zelalem *et al*., 2015). The effect of number of leaves was not significant but in other study eucalyptus aqueous leaf extract affected significantly millet (Onyewotu *et al.,* 1994) and maize (Tesfaye, 2009). The leaf area significant variation is due to the stress factor of allelophatic compounds in the leaf extract of *E. camaldulensis* (Pirdashti, *et al.,* 2009). The present study is in agreement to the findings of Desalegn and Demel (2014) of significant effect on relative elongation ratio of shoot and chlorophyll fluorescence.

Chlorophyll content, total soluble sugar, nitrogen and protein of maize leaf were significantly affected by *E. camaldulensis* aqueous leaf extract. Compared to other concentration (control, 10% and 20%), the lowest chlorophyll content were on 30% aqueous leaf extract, these results are correlated with several studies, such as Janagiraman *et al*. (2005) reported that in black gram, chlorophyll a, chlorophyll b, and total chlorophyll were reduced by 50%, 138% and 71% respectively in 20% concentrated aqueous leaf extract of *E. camaldulensis*.

In addition, Abu-Romman (2011) reported that photosynthetic pigments in *Capsicum annum* seedling were significantly and negatively affected by treatment with eucalyptus, chlorophyll a, chlorophyll b and total chlorophyll decreased with increasing the aqueous leaf extract concentration. These photosynthetic pigments of maize were reduced by allelochemical stress factor. Since, reductions in chlorophyll contents were previously reported as a result of allelochemical stress (Guo *et al*., 2006). Being the disruption of electron transport chain one of the most usual ways for affecting photosynthesis by allelochemical compounds (Gonzalez *et al*., 1998; Puri and Khara, 1991). In agreement to the present study, Jiregna (2006) found significant effect of eucalyptus aqueous leaf extract on the total soluble sugar, nitrogen and protein content of maize.

The shoot dry weight (SDW) was significantly affected by *E. camaldulensis* aqueous leaf extract (Figure 2a). In agreement with the present study, others (Guo *et al.,* 2006; Siddiqui and Zaman, 2004) had proved that eucalyptus trees reduce the growth, dry weight and yield of wheat. Using the extract from leaves and bark of eucalyptus, Puri and Khara (1991) observed similar results on *Phaseous vulgaris* total biomass. The reason could be decrease in fresh of seedling or another probable reason could be the inhibitory effect of allelochemicals in uptake of water and reduction in other physiology of the crop (Yang *et al*., 2002; Guo *et al*., 2006). However, the root dry weight has not significantly affected by eucalyptus aqueous extract. Unlike to the present study, several authors showed that, eucalyptus aqueous extract was effective to cause root growth inhibition as a consequence and has negative impact on the fresh weight and fresh weight (Siddiqui and Zaman, 2004). However the

result of current study agree with the work of Dawar *et al.* (2007) who reported that eucalyptus had insignificant effect on the root dry weight of chick-pea.

Correlation analysis revealed that the plant height had a positive correlation with most of the parameters except number of green leaves. Similar observation was reported by Desalegn and Demel (2014), while Jiregna (2006) and Mulugeta (2010) found a positive correlation between plant height, number of green leaf and grain yield. This may be due to the ceases translocation from the reserves because of environmental stress factors, particularly during grain filling and grain development period (Erick and Musick, 1979). The leaf area was found to have significant correlation with most of the morphological, physiological and biochemical traits and these results are consistent with previous report (Selamyihun, *et al*., 2005; Mulugeta, 2010). Chlorophyll contents had a positive correlation with all traits. These results are in conformity with the finding of Desalegn and Demel (2014) and obtained by Selamyihun *et al*. (2005) in wheat.

Root dry weight, girth diameter, leaf area, nitrogen and protein contents had showed positive correlation while they were insignificantly varied with number of green leaves. Similar result has been reported by many workers (Guo *et al.*, 2006; Desalegn and Demel, 2014). Leaf nitrogen had a positive correlation with chlorophyll contents. In agreement with the present study, Jiregna (2006) found strong correlation with chlorophyll content and biomass. This was expected because nitrogen is one of the constituting elements of chlorophyll. The relationship between them is linear, if the nitrogen content in the leaf is higher, naturally increases chlorophyll content, which intern increase the assimilation rate and biomass (Gonzalez *et al*., 1998).

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