Productivity and photosynthetic pigments in bell pepper plants grown in soil with biofertilizer and protected against water loss

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ABSTRACT

The search for techniques that maximize the use of water is becoming necessary for the sustainability of agriculture in semi-arid areas. In this sense, the present work aimed to evaluate the effects of soil protection against water loss and the use of bovine biofertilizer on fruit production and chlorophyll content in bell pepper plants. The experiment was conducted in the municipal district of Nova Floresta in the semi-arid region of the State of Paraíba during the period of August 2010 to February 2011. The experimental design was a randomized block design with four repetitions in a 2 × 2 × 2 factorial scheme, corresponding to soil without and with bovine biofertilizer, without and with mulch, and without and with side coating of the grooves with polyethylene film, to reduce water loss through evaporation and lateral movement of water. The results showed that the use of side coatings of the grooves led to greater production, generating productivity gains in bell pepper cultivation. The association between side coating of the grooves and mulching of the soil resulted in increased levels of chlorophyll, but the bovine biofertilizer × side coating × mulch interaction inhibited the production of chlorophyll in the plants.

Keywords: Capsicum annuum L.; chlorophyll; mulch; reduction of water loss.

INTRODUCTION

Bell pepper (Capsicum annuum L.) is among the ten most grown vegetables and is of great economic importance in Brazilian markets (Melo et al., 2017; Santos et al., 2020; Sediyama et al., 2014). Bell pepper cultivation requires a regular supply of water throughout the growing cycle, water deficiency being one of the most limiting factors to obtaining high yields (Cosic et al., 2015; Malika et al., 2019; Sezen et al., 2019).

In the semi-arid conditions of the northeastern region of Brazil, water is a resource that has become increasingly scarce. This problem, in addition to the global climate changes that are occurring, requires vegetable farmers to adopt techniques that increase the efficiency of irrigation and water use (Kuşçu et al., 2016).

Greater water-use efficiency (WUE) can be obtained with the use of crop management techniques that minimize the water loss by evapotranspiration and by lateral movement, which reduce the water content of the root environment of plants. In the case of vegetables, a practice widely used by producers is the use of ground cover, which, in addition to reducing evaporative water loss, exerts weed control (Braga et al., 2017; Liang et al., 2011; Rocha et al., 2018). Ground cover also improves the nutritional status of plants, which is reflected by the increased productivity, and thus is a practice recommended for use in semi-arid regions, where availability of water resources is limited (Braga et al., 2017; Rocha et al., 2018).

Management practices that contribute to greater retention of soil moisture near the root system of plants,
reducing lateral water loss, can be a viable alternative in bell pepper cultivation. When side coating of the planting pits in yellow passion fruit was used, Cavalcante et al. (2005) concluded that the side coating helped maintain a higher soil humidity, providing an increase in fruit production.

In addition to reducing the volume of water applied, the nutritional requirements of plants must be met in order to obtain economically viable yields. In this regard, vegetable producers apply organic inputs, such as biofertilizers, which provide nutrients to the plants, to maintain a more balanced plant nutritional status (Borges et al., 2016; Oliveira et al., 2014; Sediyama et al., 2014). As such, the objective of this study was to evaluate the effects of side coating with polyethylene film, mulching with sisal residue and application of chemically enriched bovine biofertilizer on the fruit production and chlorophyll content in bell pepper plants.

MATERIALS AND METHODS

Site description

This work was conducted in the municipal district of Nova Floresta, a semi-arid area in the State of Paraíba, Brazil, 6° 26’ 40” south latitude and 36° 12’ 04” west longitude, at an altitude of 669 m above sea level, between the months of August and February 2010/2011. Based on the Köppen classification system (Alvares et al., 2013), the climate of the region is hot and dry, with rainfall concentrated in the period from March to July.

The annual cumulative precipitation in 2010 and 2011 was 449.9 and 1036.7 mm, respectively. The total rainfall recorded during the trial period was 312.8 mm (AESA, 2020). The minimum, average and maximum temperatures in the years 2010 and 2011 were 19.6 and 19.0; 25.5 and 25.4; 32.5 and 31.8 °C, respectively (Table 1).

The soil of the experimental area, according to the criteria of the Brazilian System of Soil Classification (SiBCS) (EMBRAPA, 2013), was classified as an Eutrophic Yellow Latosol. Before the establishment of the experiment, soil samples at depths of 0-15 and 15-30 cm and samples of the manure used in the pits were collected to determine their chemical attributes (Table 2), using the methodologies suggested by Donagema et al. (2011). The soil was evaluated as well as the salinity of the saturation extract (Richards, 1954) and the physical attributes (Donagema et al., 2011), which are presented in Table 3.

Biofertilizer production

The chemically enriched biofertilizer was obtained by anaerobic fermentation of a mixture of equal volumes of fresh manure and water (not saline or chlorinated), 1% MB-4 rock powder (17.82% of Mg²⁺), 1% ash (13.61 cmol·dm⁻³ of K⁺), 1% brown sugar and 1% milk in a polyethylene biodigester with a capacity of 240 L. This container was kept in the shade and hermetically sealed for 30 days. During this preparation period, homogenization by agitation occurred every 24 h for better efficiency of fermentation (Cavalcante et al., 2019). To release the gas produced by fermentation, a hose was connected to the top of the container that, along with the other extremities, was kept submerged in a container with water to prevent air from entering.

After fermentation but prior to application to the soil, three samples of the biofertilizer were collected at the top, middle and bottom of the biodigester and diluted in water at a proportion of 1:1 for evaluation of the electrical conductivity, pH and chemical composition of the water used for irrigation (Richards, 1954), as indicated in Table 4.

Experimental design and field management

The experimental design was a randomized block design with four repetitions that adopted a 2 × 2 × 2 factorial scheme totaling 32 plots, each consisting of 21 plants, for a total of 672 pepper (Capsicum annuum cultivar Interprise) plants. The factorial corresponded to soil with and without chemically enriched bovine biofertilizer; soil with and without mulch, to reduce water

Table 1: Monthly mean values of temperature and precipitation observed in during the research period

| Month          | Minimum | Mean   | Maximum | Precipitation |
|----------------|---------|--------|---------|--------------|
| August/2010    | 17      | 22.94  | 29      | 6.6          |
| September/2010 | 18      | 24.07  | 32      | 9.4          |
| October/2010   | 19      | 26.15  | 34      | 10.0         |
| November/2010  | 20      | 26.55  | 33      | 0.0          |
| December/2010  | 20      | 26.26  | 33      | 27.2         |
| January/2011   | 20      | 25.69  | 32      | 201.6        |
| February/2011  | 21      | 26.04  | 32      | 58.0         |
| Mean/Sum       | 19.3    | 25.4   | 32.1    | 312.8        |

Source: ¹AGRITEMPO (2020) – Weather station: TRMM. 7499 – Cuité, PB. Latitude: -6.5; Longitude: -36. ²AESA (2020).
loss by evaporation with sisal residues 5 cm in thickness; and soil with and without side coating of the grooves with plastic polyethylene film, to reduce water loss by lateral movement of water.

The grooves were built with a length of 10 m and a width and depth of 40 cm. The grooves were separated by 1 m and coated with a 150-µm plastic film, as suggested by Cavalcante et al. (2005) to reduce water losses in yellow passion fruit. The grooves were then filled with the soil mix from the top layer. Afterward, the pits were opened in the grooves with dimensions of 30 × 30 × 30 cm, and 3.5 kg of cow manure was added, with 4% moisture, to raise the soil organic matter content to 4%.

The seedlings were prepared at the start of the second week in August 2010 in polyethylene trays of 200 cells using Plantmax® substrate and transplanted 28 days after sowing (DAS). The plants were spaced 1 m between rows and 0.5 m between plants, corresponding to 21 plants per plot, and the 10 middle plants of each plot were evaluated.

The chemically enriched biofertilizer, after being diluted in water (1:1), was applied manually to the soil at a rate of 10 L m⁻² (Santos, 1992) one week before transplanting and at a rate of 1 L plant⁻¹, applied to the canopy area of the tops of the plants, at 60 days after transplanting (DAT), a time that coincided with the beginning of the flowering period and increased nutritional requirements of the plants (Marcussi et al., 2004). The treatments without biofertilizer received the same volume of water as the organic liquid inputs.

### Table 2: Results of the chemical analyses as well as the soil fertility and cow manure used

| Chemical attributes | Depths (cm)       | Cow manure      |
|---------------------|-------------------|-----------------|
|                     | 0 - 15            | 15 - 30         |                  |
| pH in water (1:2.5) | 6.44              | 6.49            | 8.48             |
| OM (g kg⁻¹)         | 10.27             | 6.81            | 412.73           |
| P (mg dm⁻³)         | 25.48             | 9.56            | 668.44           |
| K⁺ (mg dm⁻³)        | 116.00            | 76.14           | 2361.20          |
| Ca²⁺ (cmol dm⁻³)    | 3.25              | 2.85            | 4.55             |
| Mg²⁺ (cmol dm⁻³)    | 1.00              | 0.85            | 6.20             |
| Na⁺ (cmol dm⁻³)     | 0.40              | 0.16            | 5.58             |
| SB (cmol dm⁻³)      | 4.95              | 4.05            | 22.37            |
| H⁺+Al³⁺ (cmol dm⁻³) | 1.40              | 1.73            | 2.31             |
| Al³⁺ (cmol dm⁻³)    | 0.00              | 0.00            | -                |
| CEC (cmol dm⁻³)     | 6.35              | 5.78            | -                |
| V (%)               | 77.95             | 70.12           | -                |

OM = Organic matter in the soil by oxidation with potassium permanganate; P, K and Na⁺ = Mehlich 1 extractor; Al³⁺, Ca²⁺, Mg²⁺ = extractor KCl 1M; SB = Sum of bases (Na⁺ + K⁺ + Ca²⁺ + Mg²⁺); CEC = Cation exchange capacity = SB + (H⁺ + Al³⁺); V = Exchangeable base saturation (100×SB/CEC).

### Table 3: Chemical attributes as well as the salinity and soil physical properties of the experimental area

| Salinity of soil | 0-15 cm | 15-30 cm | Physical attributes | 0-15 | 15-30 |
|------------------|---------|----------|---------------------|------|-------|
| EC at 25 °C (dS m⁻¹) | 1.40 | 1.70 | Soil bulk density (g cm⁻³) | 1.64 | 1.66 |
| pH               | 7.28   | 6.86   | Soil particle density (g cm⁻³) | 2.68 | 2.68 |
| Ca²⁺ (mmol L⁻¹)  | 3.20   | 5.80   | Total porosity (m³ m⁻³) | 0.39 | 0.38 |
| Mg²⁺ (mmol L⁻¹)  | 2.60   | 4.70   | Sand (g kg⁻¹) | 580 | 617 |
| Na⁺ (mmol L⁻¹)   | 1.22   | 0.98   | Silt (g kg⁻¹) | 135 | 192 |
| K⁺ (mmol L⁻¹)    | 0.91   | 0.76   | Clay (g kg⁻¹) | 285 | 191 |
| Cl⁻ (mmol L⁻¹)   | 12.50  | 5.00   | Clay disper. in water (g kg⁻¹) | 79 | 26 |
| CO₃⁻ (mmol L⁻¹)  | 0.00   | 0.00   | Index of flocculation (%) | 72.3 | 86.4 |
| HCO₃⁻ (mmol L⁻¹) | 2.00   | 0.50   | Index of dispersion (%) | 27.7 | 13.6 |
| SO₄²⁻ (mmol L⁻¹) | 0.92   | 0.79   | Water content pF (g kg⁻¹) | 8.32 | 9.57 |
| SAR (mmol L⁻¹)   | 0.72   | 0.43   | Water content pwp (g kg⁻¹) | 3.32 | 3.87 |
| ESP (%)          | -      | -      | Water available (g kg⁻¹) | 5.00 | 5.60 |

| Classification | NS | NS | Classification | SCL | SCL |
|----------------|----|----|----------------|-----|-----|
| EC = electrical conductivity; SAR = Sodium adsorption ratio = Na⁺/[(Ca²⁺ + Mg²⁺)/2]¹/²; ESP = Exchangeable sodium percentage; NS = not saline; Index of flocculation = [(Clay - Clay dispersed in water)/Clay]100; Index of dispersion = (100 – Index of flocculation); Water content pF = Water content at field capacity, tension of -0.010 MPa; Water content pwp = Water content at the permanent wilting point, tension of -1.5 MPa; SCL = Sandy clay loam; = 100 (Na⁺/CEC).
Irrigation was provided daily by the drip-irrigation method using drip tape with drippers spaced 20 cm apart at a flow rate of 4.75 L h⁻¹, which was based on the evaporation of water in a class “A” tank installed at the location of the experiment. The irrigation depth applied in all treatments was estimated based on the crop evapotranspiration (ETc), using the equation: ETc = ET₀ × Kc, which ET₀ corresponds to reference evapotranspiration and Kc to crop coefficient. The Kc values used for the culture were 0.40 at seedling setting stage; 0.40 at vegetative stage; 0.70 at flowering/fructification stage; 1.05 at full flowering stage; 0.85 at in the declining production stage (Marouelli & Silva, 2012).

**Sampling and measurements**

At 70 DAT, whole leaves were collected (the first fully developed leaf from the top) to determine the concentration of the photosynthetic pigments chlorophyll a and b as well as the total chlorophyll, as suggested by Malavolta et al. (1997) for the assessment of the nutritional status of plants. The leaves, after being collected, were immediately placed in aluminum envelopes, packed into a thermal box with ice and taken to the lab.

In the laboratory, plant tissue samples were removed from the middle third of each leaf, and the fresh mass was obtained using a precision analytical balance. The material was then macerated and packaged into containers coated with aluminum sheets. Twenty-five milliliters of 80% acetone was added, and the materials were kept cold (8 °C) for 24 h, after which they were filtered on filter paper for 5 min (Arnon, 1949).

The absorbances of the extracts were obtained using an absorption spectrophotometer at wavelengths of 646.8 (A₆₄₆.₈) and 663.2 nm (A₆₆₃.₂), with 80% acetone used as a blank. To calculate the concentrations of chlorophyll a and b and the total chlorophyll (Chl a, Chl b and Chl t, respectively), the equations described by Lichtenthaler (1987) were used.

Chlorophyll a (Chl a) = (12.25 A₆₄₆.₈) - (2.79 A₆₆₃.₂)
Chlorophyll b (Chl b) = (21.50 A₆₄₆.₈) - (5.10 A₆₆₃.₂)
Total chlorophyll (Chl t) = (7.15 A₆₆₃.₂) - (18.71 A₆₄₆.₈)

The values of the Chl a, Chl b and Chl t levels in the leaves were transformed and expressed in milligrams per gram of fresh matter (mg g⁻¹ FM).

Harvest of the fruits occurred twice weekly throughout the production cycle for calculation of the number of fruits per plant and the plant production and productivity.

**Statistical analysis**

The data were subjected to analysis of variance, and the averages between interactions were compared by the Tukey test at the 5% level of probability using the Sisvar software program (Ferreira, 2011). The averages concerning the factors isolated by the F test are conclusive between two values (Pimentel-Gomes, 2009).

**RESULTS AND DISCUSSION**

During the bell pepper growing period, the minimum, average and maximum air temperatures were 19.3, 25.4 and 32.1 °C, respectively (Table 1). According to Mercado et al. (1997), the optimal temperature for pepper growth is 25 and 30 °C. When plants are subjected to temperatures below 15 °C or above 32 °C, growth is usually slowed and yield decreases.

**Fruit production**

The chemically enriched biofertilizer and mulch with sisal residue did not exert direct effects on the productivity or on the components of production, expressed by the number of fruits and the production per plant. On the other hand, the side coating of grooves with polyethylene film for protection against lateral water movement losses resulted in an increase from 13.6 to 17.3 of the average number of fruits per plant, which represents an increase of 27.4% (Figure 1). These values are close to the range of 15.6 to 18.6 fruits per plant reported by Cosic et al. (2015) in bell pepper plants under irrigated conditions. These results are also in agreement with those of Cavalcante et al. (2005), who established that the use of this technique increases yellow passion fruit production up to 62%.

The use of the lining in the groves of the plantings, which reduces the loss of water due to lateral movement,
must have contribution to maintaining of soil moisture throughout the day. This would enable greater absorption of water and nutrients by the plants, resulting in increased production, as recorded by Braga et al. (2017) and Rocha et al. (2018) in the cultivation of melon and in bell pepper, respectively.

The superiority of the treatments with side coating of grooves, noted for their number of fruits per plant, was also observed in the individual plant production (Figure 2). According to the results, when the penetration of water on the sides of the grooves of the plantings was contained by the action of the plastic film, there was a 22.7% increase in the production of fruit per plant. The increased availability of water to the plants and better adjustment of the temperature of the soil were possibly caused by the use of the side coating of the grooves, resulting in increases in stomatal conductance, transpiration and in intercellular CO\textsubscript{2} concentrations, contributing to an increased rate of net photosynthesis of the plants, reflected positively by the production potential (Liang et al., 2011; Malika et al., 2019; Sezen et al., 2019).

The increase in the number of fruits and plant production provided by the use of the side coating of the ridges was reflected in the increase in productivity (Figure 3). The reduction of water loss by the use of the polyethylene film increased the productivity from 19.42 to 23.78 t ha\textsuperscript{-1}, providing a 22.45% gain. Such results, evidenced also by Cavalcante et al. (2005) in yellow passion fruit genotypes, indicate that the lining of the grooves of the plantings with polyethylene film improves the water use efficiency of the crops; therefore, this technique is recommended for use in horticulture, especially in semi-arid regions.

The enhanced productivity provided by the use of the side coating of the ridges with plastic film seems promising. The productivity values obtained in this work (Figure 3) are located in the ranged of 13.46 to 21.33 t ha\textsuperscript{-1}, obtained by Ashrafuzzaman et al. (2011) in assessing the use of different plastic mulches on the production of bell peppers. Despite a significant increase, the results are still well below the range of 30.9 to 34.0 t ha\textsuperscript{-1} found by Kuşçu et al. (2016) in red peppers under fertigation with nitrogen.

![Figure 1](image1.png)

**Figure 1:** Average values of the number of fruits per plant of bell pepper grown in soil without and with side coating of the grooves.

![Figure 2](image2.png)

**Figure 2:** Production of bell pepper per plant grown in soil without and with side coating of the grooves.
The use of plastic soil cover of various colors and organic materials reduces the loss of water by evaporation, increases the availability of nutrients and reduces the variability of soil moisture during the cultivation cycle, enabling greater growth of the roots, which results in increased fruit production (Kader et al., 2017).

**Leaf chlorophyll content**

Foliar levels of Chl $a$ in the bell pepper plants were influenced by the side coating of the grooves, mulching of soil and application of the chemically enriched biofertilizer (Table 5). When the side coating of grooves was used by itself (i.e., in the absence of enriched biofertilizer and soil mulch), the levels of Chl $a$ did not vary. However, in the soil without biofertilizer and with mulch, the levels of Chl $a$ increased from 0.57 to 0.70 mg g$^{-1}$ FM with use of side coating, a gain of 22.8%. This trend was also observed by Ashrafuzzaman et al. (2011), who used different types of plastics as soil cover in the same culture, which resulted in higher levels of Chl $a$.

According to Malika et al. (2019), an adequate supply of water to plants induces an increase in the levels of chlorophyll, reinforcing the effects of the side coating of the grooves and of the mulch to keep soil wetter and cooler. A study by Amer et al. (2009) showed that cucumber (*Cucumis sativus* L.) plants under irrigated conditions exhibit positive correlations between soil moisture content and Chl $a$ and Chl $b$, with an effective increase in productivity.

In the plants of bell pepper grown in the soil with biofertilizer and mulch, the level of Chl $a$ were reduced from 0.67 to 0.54 mg g$^{-1}$ FM with the use of side coating of the grooves (Table 5). Probably the higher moisture content in the soil, provided by the use of mulch and side coating of the grooves, associated with the high electrical conductivity of the biofertilizer (Table 4), increased the saline character of soil, providing a reduction in photosynthetic pigments, especially for Chl $a$ as evidenced by Melo et al. (2017) in bell pepper irrigated with saline water.

The concentrations of Chl $b$ followed the trend observed in Chl $a$ (Table 4). In plants grown soil without enriched biofertilizer, the use of mulch and side coating applied in isolation (i.e., one or the other), did not influence the levels of Chl $b$. However, when these two techniques were used together, the levels of Chl $b$ increased from 0.24 to 0.29 mg g$^{-1}$ FM, under the same condition (Table 6). Although the use of these techniques for reducing water loss is promising, the Chl $b$ values in all treatments are below the of 0.49 mg g$^{-1}$ FM obtained by Melo et al. (2017) in bell pepper plants under irrigation with non-saline water. This difference may have occurred due to climatic factors, cultivar, water availability and cultivation practices (Malika et al., 2019; Rocha et al., 2018).

The concentrations of Chl $t$ were influenced by the side coating, mulching and application of biofertilizer (Table 7). In the plants grown in the ground with side coating of grooves and mulch, the biofertilizer promoted

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**Table 5: Levels of chlorophyll $a$ in leaves of bell pepper plants grown in soil without and with side coating of grooves, mulch and biofertilizer**

| Side coating of the grooves | Soil without biofertilizer | Soil with biofertilizer |
|-----------------------------|----------------------------|-------------------------|
|                             | Without mulch              | With mulch              |
|                             | Without mulch              | With mulch              |
|                             | 0.65 Aaα                   | 0.57 Abα                |
|                             | 0.68 Aaα                   | 0.70 Aaα                |
| With                        | 0.55 Bαα                   | 0.62 Aaα                |
|                             | 0.67 Aaα                   | 0.54 Abβ                |

Means followed by the same uppercase letter in the rows and lowercase in the columns do not differ from each other by the Tukey test at 5% probability. Equal greek letters in the line do not differ from each other by the Tukey test at 5% probability. DMS = 0.11; CV = 12.94%.

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**Figure 3:** Productivity of bell pepper grown in soil without and with side coating of the grooves.
This decrease may be due to the high electrical conductivity of the enriched biofertilizer (Table 4), which provides high levels of Ca$^{2+}$, Mg$^{2+}$, Na$^+$ and Cl$^-$ (Cavalcante et al., 2019; Oliveira et al., 2014; Sediyama et al., 2014), and the largest buildup of moisture, provided by the use of all of these techniques together, could have caused a nutritional imbalance, resulting in the absorption of some nutrients in excess, such as Na$^+$ and Cl$^-$, which are toxic to plants (Lima et al., 2018). A similar situation was recorded by Corrêa et al. (2009), who concluded that high input rates of cow and poultry manure inhibit the production of chlorophyll despite raising the levels of chlorophyll in plants. For these authors, the decreases were attributed to excess nutrients provided by the inputs in the soil solution. The high electrical conductivity of the biofertilizer (11.49 dS m$^{-1}$) may have increased the concentration of salts above the tolerable limit by the plants, causing decreases in chlorophyll concentration due to increased chlorophyllase enzyme activity, which degrades chlorophyll (Santos, 2004).

In semi-arid regions, where water availability is not always sufficient to meet crop requirements, the use of technologies that allow efficient use of water is essential to maintain agricultural activity. Even with the scarcity of water resources, the lateral coating of the planting furrows with polyethylene film, combined with the use of mulch made it possible to obtain high yields of pepper. These cultivation practices are promising and can be used by pepper producers in the region.

**CONCLUSIONS**

The side coating of the grooves with polyethylene plastic film increased the production of fruit in individual plants, promoting the productivity of bell pepper cultivation.

The side coating of grooves associated with mulching in the soil without biofertilizer, increased the levels of chlorophyll $a$, chlorophyll $b$ and total chlorophyll.

When used in association with side coating and mulch, the enriched biofertilizer reduced the levels of chlorophyll in the plants.

**ACKNOWLEDGEMENTS, FINANCIAL SUPPORT AND FULL DISCLOSURE**

This work was supported by National Institute of Science and Technology in Salinity (INCTSal) and the National Council for Scientific and Technological Development (CNPq) for the financial support.

The authors declare no conflict of interest for the submitted manuscript/publication.

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