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Influence of Colored Plastic Mulch on the Leaf Calcium, Ascorbic Acid and Chlorophyll Content of Leaf Lettuce (*Lactuca sativa* L.)

Edmar N. Franquera[1*] and Renato C. Mabesa[2]

[1] Crop Science Department, Institute of Agriculture and Forestry, Tarlac College of Agriculture
Camiling, Tarlac Philippines

[2] Graduate School, University of the Philippines Los Bańos

[*]*Corresponding author's email: edmarfranquera123 [AT] gmail.com*

ABSTRACT— ***Two lettuce varieties Looseleaf and Romaine were evaluated and grown in a vegetable experimental station to determine the effect of different colored plastic mulch (silver, red, orange, yellow and green) on calcium, ascorbic acid and chlorophyll content of leaf lettuce. Results of the study showed that lettuce when grown with red plastic mulch had higher ascorbic acid content (11.78mg/100g) compared with other colored plastic mulch. However, lettuce grown with yellow plastic mulch had higher leaf calcium (777mg/l), chlorophyll a (7.92ug/ml) and chlorophyll b (11.53ug/ml) content compared with those grown with the other colored plastic mulch. The results showed that the different colored plastic mulch had significant influence on the ascorbic acid, leaf calcium and chlorophyll content of leaf lettuce.***

Keywords— *Lactuca sativa* L. leaf calcium, ascorbic acid, colored plastic mulch

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1. INTRODUCTION

Leafy vegetable such as leaf lettuce is an important crop which has been gaining more popularity among consumers. Lettuce aside from eaten as raw which is commonly used as salad vegetables may also be eaten as cooked (Katz and Weaver, 2003). Ascorbic acid which is present in lettuce its most important health compounds. One major factor which enhances the accumulation of ascorbic acid in vegetables such as lettuce is light (Western Vegetable Newsletter, 2004). The intensity and amount of light during the growing season seems to have an influence on the amount of ascorbic acid formed in vegetables such as lettuce. This is the reason why the color of the surface of the mulch may influence the light reflected to the plants thus may enhance or decrease the amount of its ascorbic acid. Ashrafuzzaman et.al, (2009) stated that the color of the mulch could affect the ascorbic acid content of chili (*Capsicum annuum* L.). Panchal et.al. (2001) also reported that black plastic mulches produced higher ascorbic acid content in chili. Production of plant tissues and enabling plants to grow better are the important roles of calcium in plants. Hassan et al. (1995) found higher levels of calcium in leaf tissues when grown in plastic reflective mulches and influences nutrient uptake and concentrations in plants. The most important pigments for photosynthesis are the chlorophylls. An important indicator of the overall condition of the plant may reflect on the amount of chlorophyll present in a plant. Chlorophyll contents determination in a plant leaf can be used to study plant status and nutritions which has an important implication in crop productions and agricultural field management (Evans, 1989, Yoder and Pettigrew-Crosby, 1995; Niinemets and Tenhunen, 1997 Zarco-Tejada et al., 2004). An indirect way of estimating the productivity of a crop is by determining its chlorophyll content and one of the indices of the phosynthetic acivity is the chlorophyll content (Larcher, 1995). The color of the mulch may have a significant effect on the concentration of chlorophyll present in a plant leaf. According to Hamide Fatemi et.al, (2013) that the chlorophyll content of *Cucurbita pepo* was increased when grown with polyethylene mulch. Thus, this study was undertaken to evaluate the effects of the different colored plastic mulch on the leaf calcium, ascorbic acid and chlorophyll content of leaf lettuce.

2. METHODOLOGY

2.1 Location of the Study

The study was carried out in University of the Philippines Los Bańos vegetable experimental station (N 14^{o} 9'49.97'' E 121^{o} 15' 1.991, Latitude 14.163882, Longitude121.250553)

2.2 Crop Establishment

Romaine and Looseleaf lettuce seeds were sown in seedling trays with a premixed soil media which compose of one part compost and two parts carbonized rice hull (CRH). Pricking was done one week after the emergence of seeds to ensure individual seedlings per hole in the seedling tray for proper growth. The seedlings were maintained inside the plastic house for three weeks before they were transplanted.

2.3 Experimental Designs and Field Establishments

The study was laid out in a split plot design following the randomized complete block design with three replications. The plastic mulches were sprayed with paint (red, orange, yellow and green) using a pressurized sprayer to ensure the uniform application of paint within the plastic mulch. Four week old lettuce seedlings were transplanted in the field with 45x20 cm spacing. Application of 5 grams per hill of 14-14-14 was done prior to transplanting. Two weeks after transplanting, 5 grams of 46-0-0 per hill was also applied.

2.4 Data Gathered

Ten representative plants were used as sampling unit collected in random per treatment for the determination of leaf calcium, ascorbic acid and chlorophyll content. For the leaf calcium content, the Atomic Absorption Spectroscopy (AAS) was used. The dye titration method following the AOAC procedures (AOAC, 1984) was used for the determination of ascorbic acid contents. For the leaf chlorophyll contents, the samples were put in the vial with 10 mL 80% acetone and were kept cool in the dark at 4°C. The absorbance of the extracts was read at wavelengths of 663 nm and 645 nm using UV2100, UVVIS Recording Spectrophotometer (Shimadzu, Japan). Arnon's equation (Arnon 1949) was used to calculate the chlorophyll contents.

2.5 Data Analysis

The data analysis was performed using the analysis of variance at 0.05 significant differences of the statistical analysis system (SAS 9.1).

3. RESULTS AND DISCUSSIONS

3.1 Ascorbic Acid Content

Variation between varieties with ascorbic acid content was highly significant (Table 1). Looseleaf produced higher ascorbic acid (10.99 mg/100g sample) as compared with Romaine (5.33 mg/100g sample). The result could be due to genetic variations between the two varieties. According to Salunkhe et.al. (1991) the ascorbic acid content of vegetables and fruits shows a wide variation among species. Differences in ascorbic acid content of vegetable cultivars could occur under different environmental conditions (Rodriguez- Amaya 2001, Lee and Kader, 2000). The ascorbic content of lettuce grown with the different colored plastic mulch showed a highly significant variation. Lettuce grown with red plastic mulch was found to have the highest ascorbic acid content (11.78mg/100g sample). This was followed by silver (10.24mg/100g sample) orange (6.44 mg/100g sample) and yellow (6.27mg/100g sample). Lowest ascorbic acid was observed in those grown with the green plastic mulch (6.09 mg/100gsample). However, this was not significantly different in those grown with yellow plastic mulch. The differences in the ascorbic acid content of lettuce grown with different colored plastic mulch could be attributed by the effect of FR/R ratio, the quantity and quality of reflected light from the surface of the colored plastic mulch (Antonious et.al., 1996). This may explain the differences in the amount of ascorbic acid found in lettuce grown with the different colored plastic mulch since mulch color reflects different patterns of wavelength of lights and different ratios of FR/R. In addition, the color of the mulch could modify the plant microenvironment and the differences in the plant microenvironment or environmental conditions could also influence the ascorbic acid content in vegetables (Rodriguez –Amaya, 2001). It has been noted in previous experiments that the level of ascorbic acid in plants such as in *Brassica rapa* var. Perviridis could be affected by the quality of light which when grown under blue and red light has higher ascorbic acid content than under white light. This could also be a possible explanation for the higher ascorbic acid content in lettuce grown with red plastic mulch since the red plastic mulch would reflect red light onto the leaves (Ohashi-Kaneko et.al. 2007). With increasing red to far red the levels of ascorbic acid increases (Bartoli et.al. 2009) However, this was contradictory with results of other studies. Li and Kubota (2009) noted that the content of ascorbic acid was unaffected by light quality. The level of ascorbic acid in vegetables has also been suggested to be regulated by phytochromes (Biringer and Schopfer, 1970; Mastropasqua et.al., (2012).

Phytochromes are involved in regulation of several cellular responses in plants including regulation of gene expressions. This could also be related to the significant differences in the level of ascorbic acid found in lettuce grown with different colored plastic mulch. Another research revealed that the concentrations of beta carotene and ascorbic acid in the roots of carrots could be modified by reflecting the right waves of color onto the plants leaves (Pons, 2003). Hence, this could also explain the differences in the ascorbic acid content found in lettuce grown with different colored mulch which was observed to be higher in lettuce grown within the red plastic mulch. It should be noted that the different color of the mulch reflect different forms of wavelength light properties (Hunt, 2003).

Table 1. Vitamin C content (mg/100g) of leaf lettuce grown with different colored plastic mulch

MULCH COLOR	VARIETY		MEAN
	Looseleaf lettuce	Romaine lettuce	
Silver	14.36^{b}	6.11^{a}	10.24^{b}
Red	19.00^{a}	4.57^{d}	11.78^{a}
Orange	8.33^{c}	4.56^{d}	6.44^{c}
Yellow	7.20^{d}	5.35^{c}	6.27^{cd}
Green	6.09^{e}	6.09^{a}	6.09^{d}
Mean	10.99a	5.33b	

Means in the same column or row followed by a common letter(s) are not significantly different at 5% level by LSD

3.2 Leaf Calcium Contents

The calcium content difference between the two varieties was highly significant. Romaine had 796.2 mg/l while Looseleaf had 677.2 mg/l. The different colored plastic mulch influences the lettuce calcium content. The highest leaf calcium content was obtained in those grown with yellow plastic mulch (792.50 mg/l.). These were followed by orange, green and silver with 777, 747 and 733.5 mg/l, respectively. Lettuce grown with red plastic mulch had the lowest calcium content with 633.50 mg/l (Table 2). One factor which could explain the differences in the leaf calcium content of the lettuce grown with the different colored plastic mulch could be the soil temperature. The higher concentration of leaf calcium found in lettuce grown with yellow plastic mulch and the lowest concentration in red plastic mulch could be attributed to the soil temperature below the surface of the mulch which could affect the plant absorption of calcium from the soil. It should be noted that the lowest soil temperature was observed within the yellow plastic mulch and the highest soil temperature was observed within the red plastic mulch. The variation in the soil temperature could be a factor for the variation in the nutrient accumulation in plants (Verdial, 2001). The nitrogen and potassium present in plants increases while the calcium and phosporus declined when the soil temperature increases which showed that the temperature within the soil could affect the nutrient levels in plants (Muller, 1991). This could explain why those grown in red plastic mulch had lower calcium content compared to those grown with yellow plastic mulch. It was observed that a higher soil temperature exhibited within the red plastic mulch and a lower soil temperature was observed within the yellow plastic mulch. A reduction in calcium contents in corn was also found with an increase in soil temperature. However this was contradictory to some reports which stated that high soil temperatures led to the increase in the uptake of nitrogen, phosphorus, calcium and boron in shoots of corn seedlings (Klein and Ferguson, 1987).On the interaction among the varieties and the colored plastic mulch, the differences were also highly significant. Romaine grown with yellow plastic mulch had the highest with 954 mg/l while in Looseleaf the highest value was observed in those grown with orange plastic mulch (827 mg/l). The lowest was produced from Romaine grown with red plastic mulch (613 mg/l).

Table 2. Leaf calcium content (mg/l) of leaf lettuce grown with different colored plastic mulch

MULCH COLOR	VARIETY		MEAN
	Looseleaf Lettuce	**Romaine Lettuce**	
Silver	651^{b}	816^{c}	733.5^{d}
Red	654^{b}	613.^{e}	633.5^{e}
Orange	727^{a}	827^{b}	777.0^{b}
Yellow	631^{c}	954^{a}	792.5^{a}
Green	723.^{a}	771^{d}	747.0^{c}
Mean	677.2b	796.2a	

Means in the same column or row followed by a common letter(s) are not significantly different at 5% level by LSD

3.3 Chlorophyll – a

The concentration of chlorophyll a was significantly higher in Romaine (8.00 µg/mL) as compared to Looseleaf (6.10 µg/mL).The highest amount of chlorophyll a was obtained within the yellow plastic mulch with (7.92 µg/mL). However, this was not significantly different with red plastic mulch (7.77 µg/mL). Lettuce grown with the green plastic mulch followed (7.13 µg/mL). Silver (6.42 µg/mL) and orange (6.02 µg/mL) plastic mulch did not differ significantly and the lettuce grown within those color of mulch had the least values of chlorophyll a concentration (Table 3).

Table 3. Chlorophyll a concentration (µg/mL) of leaf lettuce grown with different colored plastic mulch

MULCH COLOR	VARIETY		MEAN
	Looseleaf Lettuce	**Romaine Lettuce**	
Silver	5.30^{b}	7.55^{c}	6.42^{c}
Red	7.03^{a}	8.51^{b}	7.77^{a}
Orange	5.95^{ab}	6.09^{d}	6.02^{c}
Yellow	6.45^{a}	9.39^{a}	7.92^{a}
Green	5.79^{ab}	8.47^{b}	7.13^{b}
Mean	6.10b	8.00a	

Means in the same column or row followed by a common letter(s) are not significantly different at 5% level by LSD

The result indicates that the mulch color had influenced the concentration of chlorophyll a in lettuce (Panchal et.al, 2001; Yazied and Mady, 2012). The differences in the concentration of chlorophyll a could be due to the spectral distribution of light reflected from the color of the mulch. Thus, this could influence the chlorophyll concentrations in developing leaves (Bradburne et.al., 1989). On the other hand, some studies showed a non- significant effect of the color of the mulch on the chlorophyll a concentration in plants (George et.al., 2011;) Red plastic mulch produced higher concentrations of chlorophyll a compared with the other colored mulch (George et.al., 2011). Similarly in strawberry plants, the chlorophyll content was higher in red plastic mulch as compared to the other colored plastic mulch (Wang et.al., 1998).

However, this has not been the case in the present study since those grown with the yellow plastic mulch had higher chlorophyll a concentration as compared with the other colored plastic mulch. The reason might be due to the differences in the quality and quantity of light transmitted absorbed and reflected from the color of the mulch which in turn might affect the plant growth and development including its quality (Wang et.al., 1998). Another factor which might affect the chlorophyll development or pigment accumulation in plants is the temperature within the root zone or the temperature within the soil. Ruter and Ingram (1992) mentioned that chlorophyll and carotenoid levels decreased as root zone temperature increased and this is why those grown in the yellow plastic mulch had higher chlorophyll a concentration than those grown with the red plastic mulch. It was noted that red plastic mulch had a higher soil temperature than the yellow plastic mulch. Nevertheless, Ruter (1989) found that root zone temperature had no significant effect on chlorophyll a flourescence.The combined effect of varieties and the color of the mulch with chlorophyll a concentration was perceived to be highly significant. In Romaine, highest concentration was obtained within the yellow plastic mulch (9.39 µg/mL). However, in Looseleaf, the highest value was observed within the red plastic mulch (7.03 µg/mL). The lowest value was noted in Looseleaf grown with silver plastic mulch (5.30 µg/mL).

3.4 Chlorophyll – b

Differences on the concentration of chlorophyll b between the varieties were highly significant. Romaine had higher chlorophyll b concentration with 10.74 µg/mL compared to Looseleaf with 6.44 µg/mL .

Table 4. Chlorophyll b concentration(µg/mL) of leaf lettuce grown with different colored plastic mulch in Vegetable Crop Division experimental station UPLB

MULCH COLOR	VARIETY		MEAN
	Looseleaf lettuce	Romaine lettuce	
Silver	4.33^{ab}	6.73^{b}	5.53^{d}
Red	6.62^{ab}	12.59^{a}	9.60^{ab}
Orange	7.64^{ab}	7.04^{b}	7.34^{cd}
Yellow	9.33^{a}	13.73^{a}	11.53^{a}
Green	4.30^{b}	13.63^{a}	8.96^{bc}
Mean	6.44b	10.74a	

Means in the same column or row followed by a common letter(s) are not significantly different at 5% level by LSD

The differences in the chlorophyll concentrations between the two varieties could be attributed to their differences in their genetic make-up. The different colored plastic mulch significantly affected the chlorophyll b concentration of lettuce. Among the different colored plastic mulch, lettuce grown with yellow plastic mulch (11.53 µg/mL) was the highest but was not significantly different with those grown with red plastic mulch (9.60 µg/mL). This was followed by green, orange and silver with 8.96, 7.34 and 5.53 µg/mL which are not significantly different. Light quality and spectral change of solar radiation could affect or change the concentration of chlorophyll b (Zhong Xi et.al., 1999) thus, this could be the reason for the differences in the chlorophyll b concentations of the lettuce plants. A higher chlorophyll b concentrations in cucumberwas found to be induced by red light (Zhong Xi et.al., 1999) and this could be the possible reason for the higher concentrations of chlorophyll b in red plastic mulch although lettuce grown with yellow plastic mulch had higher concentration of chlorophyll b but it did not differ significantly with those grown with the red plastic mulch. Result of a study in mungbean showed that red led light source can significantly increase the concentrations of chlorophyll (Fang Min Li et.al., 2014).
The interaction between the varieties and the color of the mulch was also observed to be highly significant. In both varieties the highest concentration of chlorophyll b was obtained from those grown with the yellow plastic mulch with 13.73 µg/mL and the lowest value was obtained within green plastic mulch with 4.30 µg/mL (Table 4).

4. CONCLUSION

The result of the study revealed that the color of the mulch has an influence on the amount and content of leaf calcium, ascorbic acid and chlorophyll content in lettuce. This may enhance the potential of increasing the mineral content of lettuce using the different colored plastic mulch.

5. ACKNOWLEDGMENT

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