PROTECTED CULTIVATION IMPROVES GROWTH OF 'LOLLO ROSSA' LETTUCE UNDER CHILLING CONDITIONS IN BENGUET, PHILIPPINES

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ABSTRACT

A study was conducted in two sites (Paoay, Atok and Balili, La Trinidad) in Benguet Province, Philippines to investigate the growth and development of lettuce variety 'Lollo Rossa' through vegetative, biomass and yield, physiological, and phytochemical parameters using low tunnel and mulch (black polyethylene plastic [PEP], alnus leaves and white PEP) as mitigation strategies against cold stress. The average temperature in Atok and La Trinidad was 15 and 21 °C, respectively. In Atok, *andap* or frost was experienced on February 15 and 16, 2017 with air temperature of 1.8 and 1.5 °C, respectively. Another *andap* occurred on March 8 and 19, 2017 having an ambient temperature of 3.3 and 3.9 °C, respectively. Performance of 'Lollo Rossa' was better with the use of low tunnel compared to those grown without. Shoot length, root and shoot fresh weight, yield, shoot dry weight, and total phenolic content (TPC) of lettuce was significantly higher with the use of plastic mulch. However, the use of alnus mulch against chilling stress was ineffective. In general, white PEP was advantageous when used in Atok, while black PEP was more beneficial when used in La Trinidad. In terms of combination effect, shoot fresh weight, yield, and root dry weight were significantly high in 'Lollo Rossa' grown in Atok under white PEP and low tunnel. 'Lollo Rossa' grown in La Trinidad had significantly high root and shoot dry weight, and yield under black PEP and low tunnel.

Key words: cold stress, secondary metabolites, low tunnel, mulch

INTRODUCTION

Plants, being sessile, are inevitable to experience various abiotic and biotic stresses which include water (drought or waterlogged condition), high or low temperatures, light, air (e.g. ozone), and salinity stresses (Sayyari et al. 2013) as well as pest and pathogens (Fujita et al. 2006). Temperature changes, including diurnal and seasonal changes, can limit respiration, photosynthesis, and growth (Moynihan et al. 1995). Environmental stresses, in general, are the primary causes of crop losses worldwide, reducing about 50% average crop yields for most crops (Bray et al. 2000).

Lettuce (Lactuca sativa L.) is the most popular salad vegetable which is consumed in increasing amounts because of peoples' perception of 'healthier foods' (Llorach et al. 2008). The

Philippines produced about 3,780 mt of lettuce harvested from an area of 501 ha during the five-year crop production survey from 2010 - 2014 (BAS 2015). The average lettuce production in Cordillera Administrative Region (CAR), specifically Benguet, was 1,195 mt planted in 145 ha. Limiting factors for the production of lettuce in the northern part of the Philippines are the incidence of pests and diseases as well as various abiotic stresses such as water, nutrients and chilling temperature.

Baguio City and Benguet Province experience temperature drops during the cool season. (Luces 2014). A temperature drop of 10°C in Baguio City experienced on January 16, 2014 was associated with a temperature drop in Benguet ranging from 6.8 to 8.5°C on that day (Galacgac 2014). Frost or *andap* was also reported on December 29 and 30, 2014 with temperatures of 12.8 and 12.7 °C, respectively. The temperature was believed to have occurred below 10°C, considering other factors such as humidity, wind, water supply and overcast skies (Mamaria 2015). With such chilling temperatures, patches of farmlands suffered crop losses from frost or *andap* in some villages in Paoay, Atok, Kibungan villages of Madaymen, Taliboy-oc, Cagam-is and Masala, the village of Cada in Mankayan and in the municipality of Loo, Buguias, all of which are located in Benguet Province (Cabreza 2014). Other major crops in Benguet include cabbage, Chinese cabbage, carrots, chayote, beans, broccoli and lettuce (Batt et al. 2007).

Mitigation strategies have been identified against cold stress such as temperature acclimation or hardening, good planting schedule, proper zone or location, site exposure and slope, availability of water for sprinkler irrigation, chemical or hormone application, and protected cultivation (Decoteau 2005). Protected cultivation is a cropping technique which uses tunnels and mulches to control partially or fully the microenvironment surrounding the plant body during their period of growth to maximize yields and resource saving (Reddy 2015). With available strategies, low temperature-affected lands may be of use for crop production rather than just being idle. There are limited data on how these strategies help lettuce crops cope in the Philippines where chilling temperature occur. Therefore, this study was conducted to investigate the growth and development of lettuce using protected cultivation against chilling stress. Specifically, this study sought to determine the use of protected cultivation on the growth and development of lettuce subjected to chilling stress, and to evaluate the physiological and chemical properties of lettuce subjected to various mitigation strategies against chilling stress. This study investigated the effect of low tunnel and mulch using white and black polyethylene plastic (PEP) and alnus (Japanese alder) mulch.

MATERIALS AND METHODS

The experiment was conducted in Paoay, Atok and Balili La Trinidad, Benguet, Philippines from December 2016 to March 2017.

Plant materials. Red (Lollo Rossa) cultivar of leaf type lettuce was used in this study. 'Lollo Rossa' lettuce has an attractive leaf color from red to brown, with strongly crimpled leaves that makes a compact rosette (Koudela and Petříková 2008). Sowing was done using seedling tray with planting media composed of 1:1 coconut coir dust and carbonized rice hull. The seedling trays were maintained inside a greenhouse nursery dedicated for seedling production.

Experimental layout and statistical analysis. The required area was prepared and divided into three blocks consisting of 30 plots measuring 1 m x 5 m. The seeds were sown in seedling trays prior to transplanting. The seedlings were transplanted in the field one month after sowing with a distance of 30 cm x 30 cm between hills and rows. This spacing allowed 30 seedlings to be planted per plot. Watering after transplanting followed to prevent transplanting shock. Application of fertilizer depended on the recommended rate for the experimental area one week before transplanting. The study was carried out

J. ISSAAS Vol. 27, No. 2: 154-165 (2021)

in a 2 x 4 factorial experiment with three replications in RCBD. Data was statistically analyzed using ANOVA and treatment means were compared using the LSD at 5% level of probability.

Setup of low tunnel and mulching materials. Low tunnel. This was constructed in a 1 m x 5 m bed using four bamboo strips to make the hoop and transparent polyethylene plastic sheet was kept in place on top of the hoops using twines. The plastic sheets were twined in such a way that it could be adjusted to cover the whole tunnel from 6 pm to 6 am, then folded on one side of the furrow from 6 am to 6 pm.

Synthetic mulches. Black and white (semi- clear) PEP sheets were then placed and stretched onto the beds, covering the edges with soil to keep them in place. Holes were cut at a planting distance of 30 cm x 30 cm.

Alnus leaves as natural mulches. Fallen leaves and small branches were collected from Halsema highway, Atok, Benguet. One week after transplanting, the organic mulch was evenly distributed on the raised bed with at least 2.5 cm thickness.

Control treatment. The control treatment in this study was without mulching material.

Cultural management and harvesting. Cultural management practices like weeding, irrigation, hilling-up and application of pesticides were properly employed throughout the duration of the experiment. Harvesting was done 40 days after transplanting.

Secondary metabolite content determination. Lettuce leaves were oven-dried at 45°C until dry for the determination of total phenolic content (Velioglu et al. 1998), total flavonoid content (Zhishen et al. 1999), anthocyanin content (Wrolstad 1976) and antioxidant activity (Shimada et al. 1992).

Data collection. Data collection were various parameters related to soil and ambient temperature, vegetative growth parameters (number of leaves), biomass and yield (shoot fresh and dry weight, and yield), chilling injury (CI) index and phytochemical content (total phenolic contents [TPC], total flavonoid content [TFC], and antioxidant activity).

Laboratory thermometers were installed in plots while digital thermometer and hygrometer was used for ambient. The soil and ambient temperature and relative humidity at the experimental area were measured at 6 am, 12 noon and 6 pm, while the aerial temperature inside the low tunnel were measured at 6 am. The CI index was taken from 32 plant samples, 40 days after transplanting, using scales from 1 to 5, with 1 being normal and 5 having severe extensive necrotic areas or growth restrictions.

RESULTS AND DISCUSSION

Number of leaves. Both low tunnel and mulch factors affected the number of leaves of 'Lollo Rossa' grown in Atok and La Trinidad (Fig. 1). 'Lollo Rossa' grown in Atok and La Trinidad had more leaves when grown under low tunnel compared to those grown without. In addition, white PEP increased number of leaves in 'Lollo Rossa' grown in Atok, while black plastic mulch was effective in La Trinidad. The average number of leaves of 'Lollo Rossa' grown in Atok and La Trinidad was 11 and 14 leaves, respectively.

The improvement of plant growth and development in terms of number of leaves was observed with the use of low tunnel and mulch. The use of low tunnel was significant in increasing the number of leaves in 'Lollo Rossa'. Protected cultivation improved the number of leaves in lettuce (Santos-Filho et al. 2009). This indicates that low tunnels are advantageous in enhancing growth in lettuce because it can increase soil and tunnel temperature for the promotion of early growth and development (Hochmuth et al. 2009; Jenni et al. 2003). However, the use of alnus leaves as mulching material was not as

effective as that of the white PEP mulch in terms of number of leaves. This may be due to the lower soil temperature with the use of alnus mulch (15.7°) compared to that of white PEP (17.7°C). Organic mulches decrease afternoon temperatures which suggests that organic mulches probably act like insulators that slow down daytime conduction of heat, thus, affecting the overall soil temperature (Schonbeck and Evanylo 1998).

The effective mulching material for 'Lollo Rossa' grown in Atok and La Trinidad was the white and black PEP, respectively. The soil mean maximum temperature for Atok and La Trinidad from February to March, 2017 was 20.5 and 24.5°C, respectively. The use of white PEP or in combination with low tunnel in La Trinidad increased maximum temperature data at 27.1 and 28.6°C, respectively, of which is exceeding the upper optimal temperature limit of 25°C (DAFF 2010). However, the use of black PEP only exceeded the upper optimal temperature by 1°C. This probably explains that temperatures above optimum will have a negative effect on the plant (Lament 1993). On the other hand, heat is needed by the plants in Atok due to its cold climate. The use of white PEP alone, or in combination with low tunnel was able to increase the soil average temperature to 17.7°C and 18.2°C, respectively, against that of bare soil at 15.7°C.

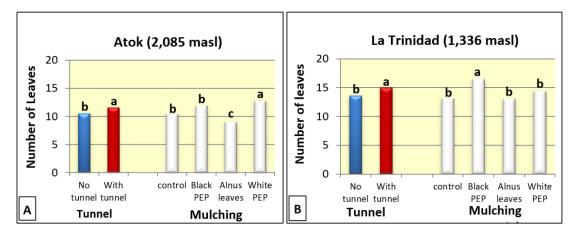


Fig. 1. Effect of low tunnel and mulching material on the number of leaves of 'Lollo Rossa' grown under chilling conditions in (A) Paoay, Atok and (B) Balili, La Trinidad, Benguet. Significant factors: main effects of low tunnel and mulch for both locations. Means with a common letter within factors in each location are not significantly different at 5% level using LSD. Legend: masl= meters above sea level.

Shoot fresh weight. Shoot fresh weight of 'Lollo Rossa' grown in both locations were significantly influenced by the interaction between low tunnel and mulch (Fig. 2). A four-fold increase in shoot fresh weight was obtained in 'Lollo Rossa' grown in Atok under white PEP, while about three-fold increase under black PEP mulch. All plants grown under bare and mulched soil further increased in shoot fresh weight, but the combination of white PEP and low tunnel gained the highest shoot weight. In La Trinidad, the use of black or white PEP mulch significantly increased shoot fresh weight of 'Lollo Rossa' by about two-fold relative to those grown in bare soil. Except for the use of white PEP and alnus mulch, plants under bare soil and black PEP mulch had significantly higher shoot fresh weight when combined with low tunnel. Overall, the use of black PEP mulch combined with low tunnel obtained the heaviest shoot fresh weight.

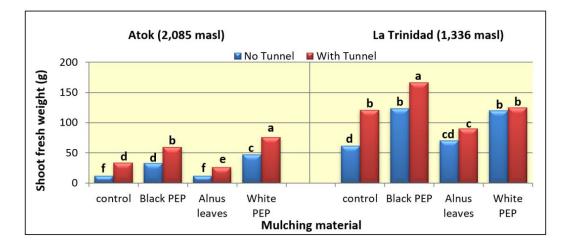


Fig. 2. Effect of low tunnel and mulching material on the shoot fresh weight of 'Lollo Rossa' grown under chilling conditions in Paoay, Atok and Balili, La Trinidad, Benguet. Significant factors: interaction effect of low tunnel and mulch for 'Lollo Rossa' grown in Atok and La Trinidad. Means with a common letter are not significantly different at 5% level using LSD. Legend: masl= meters above sea level

Aside from the capacity of mulches to increase soil temperature (Hochmuth et al. 2009; Jenni et al. 2003), low tunnels also work by trapping radiant heat during the day and delaying its loss at night (Decoteau 2005); thus, maintaining a relatively higher temperature around the plant. The control of microclimate by row cover and plastic mulches benefits the plants by enhancing crop growth and development in terms of biomass and yield, and may reduce cold damage (Decoteau 2005). This study observed that the use of mulching material increased average soil temperature ranging from 0.2 to 3.3°C higher than that in bare soil (data not shown). In addition, low tunnel alone increased morning temperature (taken during 6 AM) by 7.9% while the combination of mulching material and low tunnel increased temperature ranging from 4.7 to 13.1%. The increase in morning temperature using mulch is probably caused by the increase the air temperature around the plants (Maughan et al. 2014). Early growth and maturity of lettuce, as well as enhanced yield over the control plots were observed with the combined use of low tunnel and mulch (Jenni et al. 2003).

Shoot dry weight. The main effects of low tunnel and mulch was significant in terms of shoot dry weight of 'Lollo Rossa' grown in Atok and La Trinidad (Fig. 3). In both locations, 'Lollo Rossa' grown under low tunnel had significantly higher shoot dry weight compared to those grown without while the use of plastic mulch increased shoot dry weight of lettuce by about two-fold. However, alnus mulch was ineffective.

The use of protected cultivation such as low tunnel and mulch improved the shoot dry weight, and yield of 'Lollo Rossa' grown in Atok and La Trinidad. Protected cultivation is known to improve the dry weight of lettuce plants compared to open field (Santos-Filho et al. 2009). PEP mulches are commonly used in vegetable and fruit production (Decoteau 2005). The advantages of PEP mulches include earlier yields, better fruit quality, and higher yields. These responses may be due to enhanced soil temperature, improved water and nutrient use efficiency.



Fig. 3. Effect of low tunnel and mulching material on shoot dry weight of 'Lollo Rossa' grown under chilling conditions in Paoay, Atok and Balili, La Trinidad, Benguet. Significant factors: main effects of low tunnel and mulch for 'Lollo Rossa' grown in both locations. Means with a common letter are not significantly different at 5% level using LSD. Legend: masl= meters above sea level.

Yield. Interaction effects of low tunnel and mulch was significant on the yield of 'Lollo Rossa' grown in both locations (Fig. 4). A significant three- and four-fold increase in yield was obtained in 'Lollo Rossa' grown in Atok under black and white PEP mulch, respectively. Yield was further increased significantly in plants grown under bare and mulched soil, wherein the combination of white PEP and low tunnel gained the highest. On the other hand, the use of black or white PEP mulch increased shoot fresh weight of 'Lollo Rossa' grown in La Trinidad by about two-fold compared to those grown in bare soil. Plants under bare soil and black PEP mulch increased yield when combined with low tunnel, except for the use of white PEP and alnus mulch. Overall, the use of black PEP mulch combined with low tunnel obtained the heaviest shoot fresh weight.

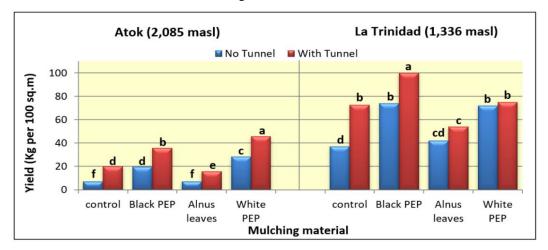


Fig. 4. Effect of low tunnel and mulching material on the yield of 'Lollo Rossa' grown under chilling conditions in Paoay, Atok and Balili, La Trinidad, Benguet. Significant factors: interaction effect of low tunnel and mulch for 'Lollo Rossa' grown in both locations. Means with a common letter are not significantly different at 5% level using LSD. Legend: masl= meters above sea level.

Yield of lettuce in Philippine setting is around 12-15 tons ha⁻¹ (Maghirang et al. 2010). However, the yield of 'Lollo Rossa' grown in Atok was 0.7 to 4.55 t/ha while those grown in La Trinidad ranged from 3.7 to 10.0 t/ha. The low yield obtained in Atok was due to the effect of chilling temperatures during the experiment where *andap* was experienced in the locality. Lower growth in 'Lollo Rossa' may suggest that it is sensitive to chilling conditions. Low temperatures were also experienced in La Trinidad, but the low yield in 'Lollo Rossa' was most likely varietal in nature. The varietal differences were also observed by Koudela and Petříková (2008) using selected cultivars of leaf lettuce. Since yield and its component characters are polygenic in nature (Meena et al. 2010) they are highly influenced by environmental factors. Temperature below optimum causes reduction in the rates of metabolism, growth and development (DAFF 2010). In addition, there is slow uptake of nutrients at low temperature, which influences the root and overall plant growth and development (Moorby and Graves 1980).

Chilling injury index. Interaction effect of low tunnel and mulch was noted on the chilling injury index of 'Lollo Rossa' grown in Atok (Fig. 5). The use of white PEP mulch significantly lowered the CI index of 'Lolo Rossa' under chilling stress in Atok. Slight to moderate CI index were observed on plants grown under white or black PEP mulch, while moderate to severe CI index from plants grown on bare soil or under alnus mulch. Except for plants grown in alnus mulch, those grown under bare soil and plastic mulch significantly lowered CI index values when combined with low tunnel. The highest benefit of low tunnel utilization was observed when combined with white or black PEP, with two-fold decrease in CI index values. In La Trinidad, no signs of chilling stress were observed on 'Lollo Rossa'.

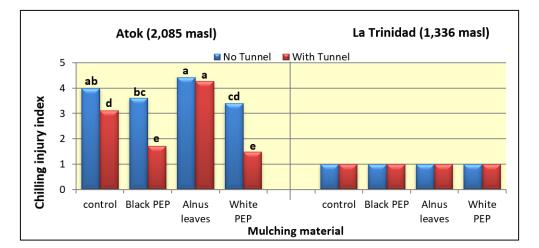


Fig. 5. Effect of low tunnel and mulching material on chilling injury index of 'Lollo Rossa' grown under chilling conditions in Paoay, Atok and Balili, La Trinidad, Benguet. Significant factors: interaction effect of low tunnel and mulch for 'Lollo Rossa' grown in Atok. Means with a common letter are not significantly different at 5% level using LSD. Legend: masl= meters above sea level.

The CI scores in this study were based on the severity of necrosis or stunting of growth. The combination of plastic mulch and low tunnel decreased CI index in 'Lollo Rossa'. A similar study showed the use of plastic mulches alone did not protect muskmelon plants from chilling injuries with seven sequential nights with temperatures between 1.6 and 1.8 °C. The combination of plastic mulches and tunnels protected the plants from chilling injuries (Jenni et al. 2003). Protected cultivation, through the use of mulch and low tunnel affected microclimate by increasing the temperature around the plants that may reduce cold damage (Faivor 2014; Maughan et al. 2014; Decoteau 2007).

Total phenolic content (TPC). Interaction effect of low tunnel and mulch was significant on the TPC of 'Lollo Rossa' grown in Atok (Fig. 6). The use of black or white PEP and alnus mulch significantly increased TPC in 'Lollo Rossa' grown in Atok relative to those grown in bare soil. Except for plants grown under alnus mulch which decreased significantly TPC when combined with low tunnel, the TPC of plants grown under plastic mulch or bare soil was not affected by low tunnel or mulching material. In La Trinidad, the main effects of low tunnel and mulching material separately affected TPC of 'Lollo Rossa'. The TPC in 'Lollo Rossa' was significantly higher when grown under low tunnel compared to those without. The use of plastic and alnus mulch increased significantly TPC in 'Lollo Rossa', where the use of white PEP obtained the highest TPC.

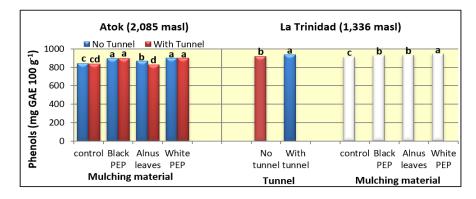


Fig. 6. Effect of low tunnel and mulching material on total phenolic content [mg gallic acid equivalent (GAE)/ 100 g] of 'Lollo Rossa' grown under chilling conditions in Paoay, Atok and Balili, La Trinidad, Benguet. Significant factors: interaction effect of low tunnel and mulch for 'Lollo Rossa' grown in Atok; significant main effects of low tunnel and mulch for 'Lollo Rossa' grown in La Trinidad. Means with a common letter are not significantly different at 5% level using LSD. Legend: masl= meters above sea level.

In this study, protected cultivation such as the use of mulch and low tunnel increased significantly the total phenolic compounds in 'Lollo Rossa' grown in Atok compared to that in bare soil, except for the use of low tunnel alone which was comparable to those grown in bare soil. Plants grown under low tunnel or the use of white PEP increased TPC relative to the control. Contrastingly, Oh et al. (2011) observed that the two lettuce cultivars, 'Baronet' and 'Red Sail', accumulated leaf phenolic concentration better when grown in open field than in high tunnel. The difference in response of lettuce may be due to the temperature at the time of the experiment. There was higher temperature of about 20°C during the conduct of the experiment of Oh et al. (2011) compared to the present study which was 15 °C. Furthermore, 'Baronet' and 'Red Sails' only obtained the highest value of TPC at about 35 and 70 mg GAE/ 100 g, while 955 mg GAE/ 100 g for 'Lollo Rossa' was noted in the present study. This implies that cold stress may have still prevailed even with the use of mulch or low tunnel.

Total flavonoid content (TFC). The interaction effect of low tunnel and mulch was significant for 'Lollo Rossa' grown in Atok and La Trinidad (Fig. 7). The use of plastic or alnus mulch significantly increased TFC in 'Lollo Rossa' grown in Atok relative to the control. The TFC further increased when combined with low tunnel. In La Trinidad, the use of plastic or alnus mulch slightly increased the TFC in 'Lollo Rossa' compared to the control. Those grown in bare soil and black PEP mulch significantly increased in TFC when combined with low tunnel, while the TFC of those grown under white PEP and alnus mulch was not affected when combined with low tunnel. The most bioactive secondary metabolites in plants are flavonoids. The biosynthesis of flavonoids is often greatly stimulated in stress-related conditions (Agati et al. 2012). Aside from environmental factors, varietal or genetic factor can influence the biosynthesis and accumulation of secondary metabolites (Yang et al. 2018). García-Macías et al. (2007) added that the intervarietal genetic variation is in general more critical than

J. ISSAAS Vol. 27, No. 2: 154-165 (2021)

environmental influences. In this study, the TFC of 'Lollo Rossa', taken 40 days after transplanting, ranged from 246.5 to 262.7 mg quercetin equivalent (QE)/ 100 g. On the other hand, flavonoid content was found to be 138.4 mg QE/ 100 g and 20.7 mg QE/ 100 g in red tissues of 'Lollo Rosso' (Ferreres et al. 1997; DuPont et al. 2000). Furthermore, 'Lollo Rosso' lettuce cultivated under UV block, UV low and UV window plastic film accumulated flavonoid averaging at 38.2, 141.2 and 182.5 QE/ 100 g, respectively (García-Macías et al. 2007).

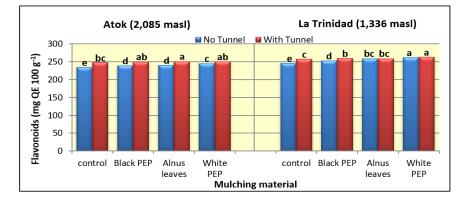


Fig. 7. Interaction effect of low tunnel and mulching material on total flavonoid content [mg quercetin equivalent (QE) 100 g⁻¹] of 'Lollo Rossa' grown under chilling conditions in Paoay, Atok and Balili, La Trinidad, Benguet. Means with a common letter are not significantly different at 5% level using LSD. Legend: masl= meters above sea level.

Antioxidant activity. Low tunnel and mulch separately affected the antioxidant activity of 'Lollo Rossa' grown in Atok (Fig. 8). The use of white PEP or alnus mulch had significantly higher antioxidant activity in 'Lollo Rossa' grown in Atok relative to those grown in bare soil. Plants grown in mulching materials significantly increased in antioxidant activity when combined with low tunnel. Antioxidant activity in 'Lollo Rossa' grown in La Trinidad was influenced separately by the main effects of low tunnel and mulching material. Antioxidant activity was significantly higher in lettuce grown under low tunnel compared to those grown without, while plastic and alnus mulch significantly increased antioxidant activity in 'Lollo Rossa' grown in Atok and La Trinidad was 80 and 87%, respectively.

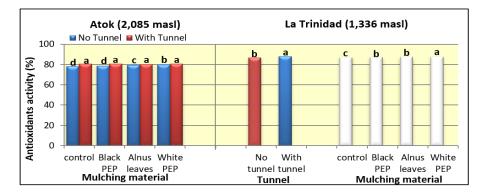


Fig. 8. Effect of low tunnel and mulching material on antioxidant activity of 'Lollo Rossa' grown under chilling conditions in Paoay, Atok and Balili, La Trinidad, Benguet. Significant factors: interaction effects of low tunnel and mulch for 'Lollo Rossa' grown in Atok; main effects of low tunnel and mulch for 'Lollo Rossa' grown in La Trinidad. Means with a common letter are not significantly different at 5% level using LSD. Legend: masl= meters above sea level.

The use of low tunnel alone or combined with mulch significantly increased the antioxidant activity of 'Lollo Rossa' grown in Atok as compared to the control. In La Trinidad, there was an increase in antioxidant activity from 'Lollo Rossa' grown in low tunnel. In contrast with antioxidant activities recorded from Atok and La Trinidad, two lettuce cultivars, 'Baronet' and 'Red Sail', accumulated phenols and antioxidants better when grown in open field than in high tunnel. In addition, antioxidant capacity followed the trend in the accumulation of total phenols (Oh et al. 2011). In this study, there was an increase in total phenolic content with increasing antioxidant activity. This trend was followed by 'Lollo Rossa' grown in both locations under low tunnel, white PEP or alnus mulch alone. Previous studies showed a larger overall effect for some combinations of antioxidants (Uri 1961).

CONCLUSION

The use of low tunnel or mulching material was beneficial in 'Lollo Rossa' on TPC, TFC, and antioxidant activity grown in Atok and La Trinidad, Benguet, while the number of leaves, shoot fresh and dry weight, yield, chilling injury was in Atok conditions only. In general, white PEP mulch was advantageous when used in Atok, while black PEP mulch was more beneficial when used in La Trinidad. Overall, the use of alnus mulch against chilling stress was ineffective.

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