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REGULAR ARTICLE

Influence of winter stress and plastic tunnels on yield and quality of spinach, pak choi, radish and carrot

Santosh Shiwakoti[1, 2], Valtcho D. Zheljazkov [1, 2]* and Vicki Schlegel[3]

[1]University of Wyoming, Sheridan Research and Extension Center, 3401 Coffeen Avenue, Sheridan, WY 82801, [2]Oregon State University, Department of Crop and Soil Science, 3050 SW Campus Way, Corvallis, OR 97331, [3]University of Nebraska – Lincoln, Department of Food Science and Technology, 1901 N 21st Food Innovation Center, Lincoln, NE 68588

ABSTRACT

Plastic tunnels are the viable options for the successful production of cold-tolerant vegetables during cold months of the milder climate regions. However, growing vegetables in northern climates can be a challenge because of long and severe winters. Therefore, we conducted a plastic tunnels study on carrot, radish, spinach, and pak choi in northern Wyoming, USA to explore the viability and quality of the vegetable production under winter stress. The objective was to quantify the effect of different season extension methods on the produce yield, its total phenol content, and antioxidant activity. The experiment consisted of three tunnel systems: high tunnel (Ht), low tunnel (Lt), and low tunnel within high tunnel (LtHt). We were able to harvest vegetables in the freezing November of Wyoming. Spinach, and pak choi had markedly higher yield in LtHt (6,410 kg ha^{-1} spinach and 20,644 kg ha^{-1} pak choi) than Ht (4,574 kg ha^{-1} for spinach and 12,076 kg ha^{-1} for pak choi) and Lt (3,253 kg ha^{-1} spinach and 8,242 kg ha^{-1} pak choi). The concentrations of nutrients in the vegetables weren't affected by the tunnel systems. Greater antioxidant activity of pak choi was evident in Ht than in Lt and LtHt. This experiment demonstrated that challenges of severe winter for growing cool-season vegetables can be mitigated largely by opting for LtHt rather than Lt or Ht alone.

Keywords: High tunnel, Low tunnel, Spinach, Pak choi, Carrot

INTRODUCTION

Wyoming has long and harsh winters, frequent windy conditions, short frost-free periods (120-125 days), and high elevation (945 m to 4207 m above sea level) (Zheljazkov et al., 2014). These conditions are not conducive to produce most vegetables. Growing cool-season vegetables in the late fall/winter is not possible without additional protection in the region. In addition to that, research on season extension of vegetable production in severe winter stress climates like Wyoming is limited. Therefore, we expect this study will open new opportunities for cool-season vegetables production in the Wyoming and in the similar climatic zone around the globe.

High tunnels vegetables growing system produce better-quality vegetables and fetch more price on the market than the produce grown conventionally i.e. in open field-growing system. Recently, the use of high tunnels for extending the growing season has increased dramatically (Lamont, 2005, 2017; Lamont et al., 2003; Waterer, 2003). Constructing plastic tunnels above the beds can change the microclimate by increasing the temperature inside the tunnels, blocking the wind, and maintaining humidity (Lodhi et al., 2013; Shiwakoti et al., 2016). These tunnels enhance some plant physiological activities resulting in higher crop yields (Lodhi et al., 2013). Similarly, high tunnels provide a protective environment for crop production throughout the year. In addition to that, yields and quality of crops during the normal growing season largely improve under tunnels system than under open-field production system (Wells and Loy, 1993; Wells, 2000). High tunnels have performed well in the United States and are used in the production of quality cut flowers (Lamont, 2009; Upson, 1998; Wien, 2009), tomato (*Solanum lycopersicum*) (Jett, 2004), strawberry (*Fragaria ×ananassa*) (Kadir et al., 2006), and even small-statured fruit trees (Lang, 2009). Successful production of lettuce (*Lactuca sativa)* in high tunnels in Alaska was reported by Rader and Karlsson (2006). Similarly, Blomgren and Frisch (2016) and Lang (2009) reported that using low

***Corresponding author:**
VD Zheljazkov, Oregon State University, Department of Crop and Soil Science, 3050 SW Campus Way, Corvallis, OR 97331, USA.
E-mail: Valtcho.Jeliazkov@oregonstate.edu

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tunnels was advantageous to achieve higher productivity during cold seasons when the crop needed protection from frost and low temperature. The preceding claim was corroborated by the study of Hecher et al. (2014) who demonstrated successful production of lettuce and spinach (*Spinacia oleracea L.*) under different type of plastic tunnels in severe cold season of New Mexico state. According to Hecher et al. (2014), a single and double layer high tunnels were adequate for protecting growing crops, easy to build, and had high positive returns. Therefore, we hypothesized that: (i) by using the high tunnels and low tunnels, farmers of northern Wyoming could grow vegetables in the late fall, and (ii) since different tunnels height would have different amount of heat entrapment, tunnel within a tunnel will trap more heat and will provide more suitable microclimate than high tunnel or low tunnel only.

The aim of this study was to assess three different extended season production systems and to explore the viability of cool-season vegetables production throughout the winter months in northern Wyoming. Cultivation conditions can be manipulated to maximize the antioxidant capacity of crops as well (Carey et al., 2009) and subsequently, crops grown under different types of tunnel system could exhibit differential antioxidant activity. Thus, in addition to the above-mentioned objective, exploring antioxidant activity and phenolic content of vegetables under different tunnels was our second aim. We tested the vegetbales for yield and quality under three-different types of plastic tunnel: (i) low tunnel (Lt), (ii) high tunnel (Ht), and (iii) low tunnel within the high tunnel (LtHt). Carrot (*Daucaus carota* L.), radish (*Raphanus sativus* L.), pak choi (*Brassica rapa* L. subsp. *chinensis*), and spinach (*Spinacia oleracea* L.) were tested in this experiment under the three tunnel systems. Although Ht and Lt are common season extension methods, LtHt is a novel approach and to our knowledge, no previous studies had studied cool season vegetables under these season extension methods together.

MATERIALS AND METHODS

Experimental site and tunnels design

The experiment was conducted at the Sheridan Research and Extension Center (ShREC), Sheridan, Wyoming (44°45.686' N, 106o55.479' W, 1170 m elevation above sea level). The soil type at the experimental plots was Wyarno clay loam, alluvium that was derived from shale. The experimental site was located at low (0–3%) slope. In the fall of 2011, field was plowed at 25 cm depth and then disked and harrowed in the spring. Low beds (12 cm high, 91 cm wide, and 21 m long) were prepared using a bed shaper (Press- pan type). The same machine simultaneously laid a drip-tape irrigation tube at 2-3 cm soil depth, and the beds were covered with black plastic mulch.

A round-style high tunnel was constructed using single layer of 6-mil (0.153 mm) greenhouse film (FarmTek, Dyersville, IA). The high tunnel was 6 m wide, 3.6 m high, and 22 m long. Low tunnels were constructed using 92% light transmission greenhouse grade film and galvanized hoops (1.89 m wide X 0.91 m high) (Growers Supply, Dyersville, IA). Six raised beds were prepared and divided into subplots of 1.5 m by 0.91 m plots.

Experimental methods

Of the six beds, two beds were inside the Ht without Lt (i.e. Ht treatment of this study) and two beds were in Lt under Ht (i.e. LtHT treatment of this study). The two beds outside the Ht were in Lt only (i.e. Lt treatment of this study). The treatment layout are shown in Fig.1. The design of the plots was completely randomized design within a block (CRD) with two replications of each treatment. In each treatment, each bed was divided into 10 subplots for the planting of the crops, with 10 plants per subplot. Among the 10 crops, five were herbs and five were vegetables. Herbs results were published (Shiwakoti et al., 2016). Among the five vegetables, lettuce died in the early stages, so we continued with four vegetables. The studied vegetables were: carrots (cv. Red Core Chantenay), radish (cv. French Breakfast), pak choi (cv. Pechay), and spinach (cv. Flamingo). The seeds of radish, carrot, and pak choi were purchased from W. Atlee Burpee & Co (Warminster, PA, USA) and spinach seeds from Johny's Selected Seeds (Fairfield, Maine, USA). Seeds were sown directly for all crops. Carrot seeds were sown on August 3, 2014; radish, pak choi, and spinach on September 4, 2014. Fifteen grams of 14%-14%-14% (N, P_2O_5, K_2O) controlled release fertilizer (Sun Grow Horticulture, Agawam, MA, USA) were applied once per individual plant that also supplied micronutrients. Carrot and radish were harvested on October 31st, 2014, and spinach and pak choi were harvested on November 11th, 2014. The produce was harvested only once and after more than 90% of vegetables attained optimum marketable quality.

Fig 1. Experiment site. Black, blue, and orange arrows are indicating vegetables production under low tunnels, high tunnels and low tunnel within high tunnels respectively.

Maximum-minimum thermometers (PRIMEX, Lake Geneva, WI) and tensiometers (Hydrofarm, Petaluma, CA) were installed in each treatment and the daily minimum and maximum air temperatures inside tunnels were recorded at 8 A.M daily.

Nutrient content analysis

Samples of harvested crops were dried in an oven at 65^o C for 48 hours. The samples were sent to the American Agricultural Laboratory, INC. (formerly Olsen Lab), McCook, Nebraska, for nutrient content analysis. Nitrogen (N) was analyzed and determined with a LECO TruSpec CN (LECO Corp 2006) and Official Methods of Analysis (1995), and all other nutrients were extracted according to Huang et al. (2004) and CEM Corporation operation manual (CEM Corporation, 1999) and measured by inductively coupled plasma (ICP) spectrometry. Average nutrients accumulations in harvest were calculated from the product of average yield of edible parts and average nutrient concentrations in dried plant tissue.

Total phenols and antioxidant capacity analysis

Samples of harvested crops were packed in boxes with dry ice and shipped to the Small Molecule Analysis Lab-FST (SMALL), University of Nebraska-Lincoln, Lincoln, NE (USA). Samples were divided into three, and triplicate analysis was performed. We used 10 ml of 50:50 methanol: water for 1 hour to extract the samples (5 g). The homogenates were centrifuged until a pellet was formed, and the supernatant was clear.

The Folin-Ciocalteau micro method determined total phenols as described by Singleton and Rossi (Singleton and Rossi, 1965). Briefly, a sample portion (100 μL) was combined with 100 μL of Folin-Ciocalteau reagent and 4.5 mL of nanopure water. After 3 minutes of shaking of the samples at room temperature, 0.3 mL of 2% (w/v) sodium carbonate was added followed by a reaction time of 2 h at room temperature with intermittent shaking. Detection of the phenols was achieved with a UV-Vis spectrometer (Beckman Coulter, Brea, CA, USA) at a wavelength of 760 nm. A standard calibration curve using gallic acid was plotted to measure the concentrations. Total phenols were expressed in mg gallic acid/g red bean powder in mean +/- standard deviation. The extracted supernatant was also investigated for antioxidant capacity using the oxygen radical absorbance capacity (ORAC) method (Huang et al., 2002). Specific details regarding this method were previously described (Zheljazkov et al., 2013, 2015). The antioxidant capacity of the vegetables was measured using Trolox (a water derivative of Vitamin E) as a standard. The results were expressed as μmol Trolox/g red bean powder in mean +/- standard deviation.

Statistical analysis

Analysis of variance (ANOVA) of edible part yields and nutrients accumulation in plant tissue was performed on three treatments. Tukey-Kramer HSD (honest significant difference) was used to separate means of treatments that were significant. The treatments were considered significant at 95% confidence level (α=0.05) using SAS 9.6 software (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

Crop yield and nutrient contents

The temperature inside Ht and Lt were higher than the outside air temperature and overall provided a better environment for growing crops, especially when the temperatures fell during the winter. We observed highest minimum daily temperatures in the LtHt, followed by Ht, Lt, and outside temperature (Fig. 2). However, maximum daily air temperatures were greater in Ht followed by LtHt, Lt, and outside temperatures (Fig. 2).

Yields of radish, pak choi, or spinach were largely impacted by the three treatments (Lt, Ht, and LtHt) (Table 1). Except for radish, yields were highest in LtHt. Radish yield was highest in Lt (5,207 kg ha^{-1}) and lowest in LtHt (2,724 kg ha^{-1}); carrot yield was highest in LtHt (4,340 kg ha^{-1}) and lowest in Lt (3,784 kg ha^{-1}); pak choi yield was highest in LtHt (20,644 kg ha^{-1}) and lowest in Lt (8,242 kg ha^{-1}), and spinach yield was highest in LtHt (6,410 kg ha^{-1}) and lowest in Lt (3,253 kg ha^{-1}) (Table 1). Carrot yields were not significantly different among the treatments. Higher temperatures inside LtHt and Ht may have contributed to higher yields of pak choi and spinach. Minimum temperatures were usually below 5^o C and sometimes below 0^o C in Lt, whereas minimum temperatures were 2-5^o C higher in Ht or LtHt than Lt in most of the days during the growing season (Fig. 2). This result is in line with the study conducted in Pacific Northwest of USA (Borrelli et al., 2013), that reported greater spinach under plastic tunnels with warmer temperature than the tunnels with lower temperature. The latter author also attributed greater spinach production in these tunnels due to low differences between maximum and minimum temperatures inside the tunnels (Borrelli et al., 2013), which could be the probable reason for having greater spinach yield in LtHt than in Lt in this study. These results were anticipated, as the tunnels not only increase air temperature inside tunnels but also moderate low-temperature extremes in the soil by increasing and maintaining the temperature inside the soil (Lamont, 2005; Wells and Loy, 1993). Similarly, Libik and Siwek (1994) and Carey et al. (2009) mentioned that plastic cover increases the air temperature as well as soil temperature

Table 1. Average yield of edible parts (kg ha^{-1}) and accumulation of nutrients (kg ha^{-1}) in radish, carrot, pak choi, and spinach in three-season extension methods

Treatments[1]	Yield[2]	N[3]	P	K	S	Zn	Ca	Mg	Fe	Mn	Cu	B
Radish												
Lt	5207^{a}	169^{a}	31^{a}	522^{a}	25 ^{a}	0.27^{a}	28^{a}	14^{a}	3.07^{a}	0.12^{a}	0.11^{a}	0.18^{a}
Ht	2829^{b}	97^{b}	15^{b}	279^{b}	14 ^{b}	0.11^{ab}	21^{ab}	8^{b}	1.61^{ab}	0.01^{b}	0.02^{a}	0.08^{b}
LtHt	2724^{b}	98^{b}	14^{b}	291^{b}	15 ^{b}	0.11^{b}	17^{b}	7^{b}	1.36^{b}	0.01^{b}	0.13^{a}	0.08^{b}
P>F	**	*	**	*	*	*	*	**	*	**	NS	**
Carrot												
Lt	3784^{a}	47^{a}	13^{a}	128^{a}	3 a	0.08^{a}	5^{a}	6^{a}	0.82^{a}	0.53^{a}	0.02^{a}	0.12^{a}
Ht	4144^{a}	57^{a}	14^{a}	154^{a}	4 a	0.11^{a}	13^{a}	8^{a}	0.9^{a}	0.06^{a}	0.03^{a}	0.13^{a}
LtHt	4340^{a}	59^{a}	15 ^{a}	154^{a}	5 a	0.14^{a}	15^{a}	8^{a}	1.25^{a}	0.55^{a}	0.03^{a}	0.18^{b}
P>F	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*
Pak choi												
Lt	8242^{b}	498^{b}	66^{b}	836^{b}	73^{b}	0.38^{b}	176^{c}	0.35^{a}	2.53^{a}	0.34^{b}	0.05^{a}	0.31^{b}
Ht	12076^{b}	729^{ab}	92a^{b}	978^{b}	113^{b}	0.51^{b}	315^{b}	0.54^{a}	6.52^{a}	0.53^{b}	0.07^{a}	0.42^{b}
LtHt	20644^{a}	1170^{a}	165^{a}	2024^{a}	223^{a}	0.82^{a}	524^{a}	0.86^{a}	3.51^{a}	0.96^{a}	0.11^{a}	0.89^{a}
P>F	**	*	*	**	**	*	**	NS	NS	*	NS	*
Spinach												
Lt	3253^{b}	176^{b}	23^{b}	373b	12^{b}	0.18^{b}	17^{b}	35^{c}	2.51^{a}	0.15^{a}	0.03^{a}	0.12^{b}
Ht	4574^{b}	249^{b}	28^{b}	518b	18^{ab}	0.21^{b}	29^{b}	53^{b}	1.59^{a}	0.26^{a}	0.04^{a}	0.13^{b}
LtHt	6410^{a}	348	39^{a}	806a	24^{a}	0.43^{a}	40^{a}	77^{a}	3.01^{a}	0.32^{a}	0.06^{a}	0.18^{a}
P>F	**	**	**	**	**	*	*	**	NS	NS	NS	*

Mean separation within columns by Tukey-Kramer HSD test at $p < 0.05$. NS, *, ** indicate nonsignificant and significant at $p < 0.05$, and 0.01 respectively.
[1]Lt, Ht, and LtHt denote the treatments low tunnel, high tunnel, and low tunnel within high tunnel respectively.
[2]yield of marketable edible parts of vegetables.
[3]N=Nitrogen, P= Phosphorus, K= Potassium, S= Sulfur, Zn= Zinc, Ca= Calcium, Mg= Magnesium, Fe= Iron, Mn= Manganese, Cu= Copper, and B= Boron

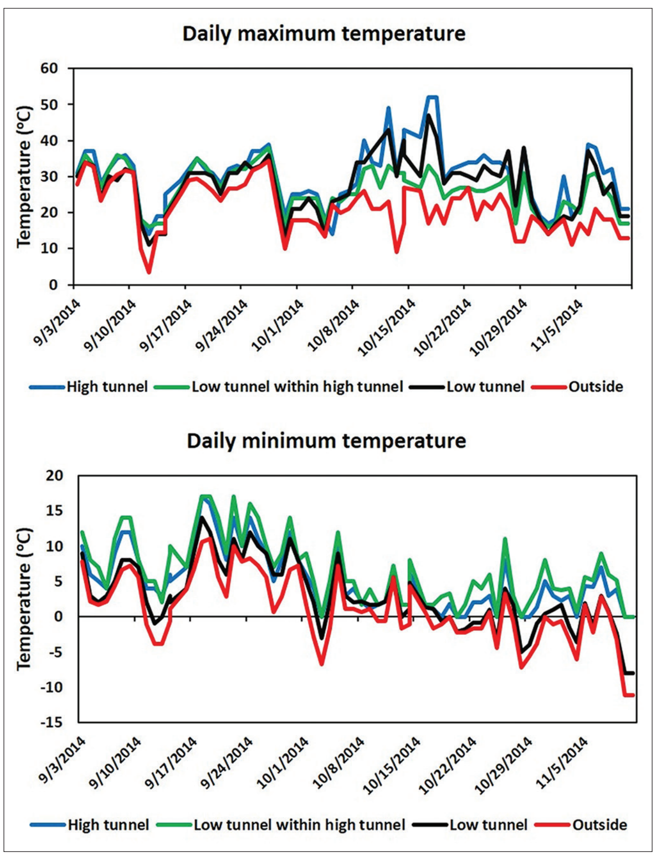

Fig 2. Daily maximum and minimum temperature in late fall of 2014 inside high tunnel, low tunnel and low tunnel within high tunnel.

and promotes crops growth and development. Soltani et al. (1995) and Nennich et al. (2004) demonstrated that tunnels are beneficial as they create a favorable microclimate for some crops. In northern Wyoming, temperatures during the growing season of cool-season crops are of vital importance as they generally fall below 0^{o} C in late fall.

The accumulation of nutrients in radish [nitrogen (N), phosphorus (P), potassium (K), sulfur (S), zinc (Zn), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), and boron (B)] were significantly different among the treatments except for copper (Cu) (Table 1). Highest nutrient accumulation of radish was observed in Lt. Similarly, some nutrient accumulation of pak choi and spinach were significantly different among treatments. Magnesium, Cu, and Fe accumulation were not significantly different in pak choi, whereas Fe, Mn, and Cu accumulation were not significantly different in spinach (Table 1). Compared to radish, nutrient accumulation of pak choi was highest in LtHt followed by Ht and Lt, perhaps because of higher temperatures inside LtHt. Low-temperature stress is one of the limiting factors for nutrient uptake and affects nutrient accumulation by crops (Nagasuga et al., 2011; Rhee and Gotham, 1981; Shimono et al., 2007). Radishes were harvested in October before continuous low-temperature stress began, and thus nutrient contents accumulation were not highest in LtHt. Nutrient concentrations of all

Table 2: Average concentrations of nutrients (mg g⁻¹) in radish, carrot, pak choi, and spinach in three-season extension methods

Treatments[1]	N[2]	P	K	S	Zn	Ca	Mg	Fe	Mn	Cu	B
Radish											
Lt	32	6	100	5	0.05	5	3	0.59	0.02	0.02	0.03
Ht	34	5	99	5	0.04	8	3	0.57	0.02	0.01	0.03
LtHt	36	5	106	5	0.04	6	3	0.50	0.02	0.09	0.03
Carrot											
Lt	12	3	33	1	0.02	3	2	0.20	0.01	0.01	0.03
Ht	13	3	37	1	0.03	3	2	0.23	0.01	0.01	0.03
LtHt	14	3	35	1	0.03	3	2	0.28	0.01	0.01	0.03
Pak choi											
Lt	60	8	102	9	0.05	21	6	0.29	0.04	0.01	0.04
Ht	60	8	81	9	0.04	26	6	0.51	0.04	0.01	0.04
LtHt	57	8	98	11	0.04	25	5	0.18	0.04	0.01	0.04
Spinach											
Lt	54	7	114	4	0.05	6	11	0.81	0.05	0.01	0.03
Ht	54	6	113	4	0.04	6	12	0.35	0.04	0.01	0.03
LtHt	54	6	126	4	0.05	6	12	0.47	0.05	0.01	0.03

[1]Lt, Ht, and LtHt denotes the treatments low tunnel, high tunnel, and low tunnel within high tunnel respectively.
[2]N=Nitrogen, P= Phosphorus, K= Potassium, S= Sulfur, Zn= Zinc, Ca= Calcium, Mg= Magnesium, Fe= Iron, Mn= Manganese, Cu= Copper, and B= Boron

Table 3: Total phenol (mg g⁻¹) and ORAC[1] (μmol Trolox Equivalent g⁻¹) of radish, carrot, pak choi, and spinach in three-season extension methods

Treatment[2]	Carrot		Radish		Pak Choi		Spinach	
	TP[3]	ORAC	TP	ORAC	TP	ORAC	TP	ORAC
Lt	0.04[a]	1.46[a]	0.41[a]	5.12[a]	0.42[a]	19.21[b]	0.31[a]	23.26[a]
Ht	0.22[a]	1.63[a]	0.41[a]	6.33[a]	0.41[a]	29.32[a]	0.51[a]	25.31[a]
LtHt	0.04[a]	1.93[a]	0.42[a]	5.23[a]	0.53[a]	14.19[b]	0.23[a]	28.14[a]

Mean separation within columns by Tukey-Kramer HSD test at $p < 0.05$.
[1]ORAC = Oxygen radical absorbance capacity expressed as micromoles per gram of trolox equivalents fresh weights.
[2]Lt, Ht, and LtHt denotes low tunnel, high tunnel, and low tunnel within high tunnel respectively.
[3]TP: Total phenols expressed in mg g⁻¹

vegetables did not differ between the treatments (Table 2). Among the nutrients, the concentration of K was a lot higher than any other nutrient in all of the crops. The average K concentrations in pak choi were 10 times higher than the K concentrations reported by Zhao et al. (2009). Zhao et al. (2009) experiments were conducted under optimal growing conditions in field and in a greenhouse.

Total phenols and antioxidant capacity

Total phenols of radish, carrot, pak choi, and spinach were unaffected by the different treatments of our study (Table 3). Carrot and spinach had higher total phenols in Ht than in the other treatments, whereas radish and pak choi had higher total phenols in LtHt than in the other treatments. Zhao et al. (2009) reported 10 times greater concentration of total phenols in pak choi than the concentrations determined in this experiment. Nitrogen deficiency, different chemotype, insect attack, and pesticide application may have caused the abnormally elevated level of total phenols in the experiment of Zhao et al. (2009). In addition, the use of different standards to determine total phenols and different treatments than in this experiment may have also contributed to different amounts of total phenols between this and the experiments of the latter authors. The latter authors had used chlorogenic acid as a standard whereas gallic acid was used in this experiment.

Antioxidant activities of radish, carrot, and spinach were not significantly different, but oxygen radical absorbance capacity (ORAC) values of pak choi were significantly higher in Ht (28.56 μmol Trolox Equivalent g⁻¹) than in Lt (19.26 μmol Trolox Equivalent g⁻¹) and in LtHt (14.08 μmol Trolox Equivalent g⁻¹). A positive correlation between total phenolic content and ORAC had been documented by many studies (Eberhardt et al., 2005; Howard et al., 2002; Proteggente et al., 2002; Reyes-Carmona et al., 2005). However, in this study, we did not observe such relation between ORAC and phenolic content as ORAC of pac choi were greater in HT than in LtHt. The ORAC of spinach from this study was double than the ORAC obtained by Cao et al. (1996). Wang et al. (2002) reported that strawberries grown on raised bed with black plastic mulch had markedly greater antioxidant capacity than on flat open bed and this could be attributed to the greater ORAC value of spinach in our study than that in the study by Cao et al. (1996). However, the spinach ORAC of this study was still five-fold less than that reported by Pant et al. (2009). The latter authors used vermicompost which may have contributed to the high ORAC values of spinach as compost in rooting media increases the antioxidant capacity of crops in a greenhouse (Wang and Lin, 2003).

CONCLUSIONS

This experiment demonstrated that carrot, radish, pak choi, and spinach can be grown successfully in northern Wyoming and perhaps, in the similar climatic regions around the world, with the help of season extension methods. Among the tested three-season extension methods, low tunnel within the high tunnel (LtHt) was the most promising season extension method. In northern Wyoming, high tunnels or low tunnels are effective enough for growing vegetables, if the vegetables can be harvested in or before October, but to get produce after October, a LtHt set up would be necessary. However, the higher minimum temperature inside LtHt during severe winter did not play a role in the quality of produce. This experiment offers optimism for cool-season vegetable farming in northern Wyoming and other similar regions.

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Author's contribution

Valtcho D. Jeliazkov, the corresponding author, designed the research plan and organized the study. Santosh Shiwakoti performed the experiment, collected the data, and did statistical analysis. Vicky Schlegel did the chemical analysis of the studied vegetables. All contributed to the writing of the manuscript.

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