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Evaluating the Potential of Protected Cultivation for Off-Season Leafy Vegetable Production: Prospects for Crop Productivity and Nutritional Improvement

Srinivasan Ramasamy[1], Mei-Ying Lin[1], Wan-Jen Wu[2], Hsin-I Wang[2] and Paola Sotelo-Cardona[1]*

[1] *Safe and Sustainable Value Chains Flagship Program, World Vegetable Center, Tainan, Taiwan,* [2] *Healthy Diets Flagship Program, World Vegetable Center, Tainan, Taiwan*

The effects of different protective structures on horticultural and nutritional yield of amaranth and water spinach were studied in three seasons of 2020–2021 in Taiwan. The number of people that can receive recommended dietary intake of iron and β-Carotene from vegetables grown under different production conditions was also estimated. The yield of white and red amaranths was consistently better (7.68–19.70 t/ha) under pink poly-net house in all the seasons, but the yield of water spinach was consistently better under white poly-net house (16.25–20.88 t/ha). Spider mite (fall & spring) and aphid (winter) infestation was mostly observed on all crops under poly-net houses. Neoxanthin, lutein and β-carotene were almost two-fold higher in red amaranth harvested from poly-net houses than open field. Based on the RDI values, β-Carotene supply to both men and women (14+) was consistently higher in all crops produced under pink ploy-net houses in all seasons, except for white amaranth produced under white poly-net house during winter. Its supply to 64,788 more men and 83,298 more women was estimated for red amaranth harvested from pink poly-net house than other production conditions. α-carotene was 2–3 fold higher in amaranths and water spinach harvested from poly-net houses than open field. The iron content of the amaranths was lower in poly-net houses (234.50–574.04 g/ha) than open field (645.42–881.67 g/ha) in the fall, but its supply from pink poly-net house was comparable with open field in the winter. However, pink poly-net house was the highest iron supplier from water spinach (323.90 g/ha) in the winter, which was estimated to provide iron to 19,450–22,939 more men and women than other production conditions. Both poly-net houses were the sole supplier of iron through amaranths in the spring, with pink poly-net house supplying iron to 2,000–5,000 more men and women. Thus, protected cultivation not only leads to more marketable yields but also results in higher quantities of health promoting nutrients. Hence, pink poly-net house may be considered to produce more nutritious vegetables, especially during the off-season to bridge the gaps in the seasonal variations in vegetable consumption, besides providing better income opportunities to the smallholder farmers.

Keywords: colored net structures, off-season production, nutritious vegetables, iron, carotenoids, amaranth, water spinach

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***Correspondence:**
Srinivasan Ramasamy
srini.ramasamy@worldveg.org

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INTRODUCTION

Leafy vegetables are an important component in the farming systems as well as in the diets of people in Asia and Africa. Various leafy vegetables such as leafy brassicas (Chinese cabbage, pak-choi, kale, mustard, etc.), amaranth, water spinach, Malabar spinach, jute mallow, chayote, spider plant and African nightshade are grown in different parts of Asia and Africa, and they are mostly considered as "traditional" vegetables, since they are a part of alimentary traditions and cultural identity (Towns and Shackleton, 2018). These vegetables play an important role among the smallholder farmers, since they are a source of food and nutritional security, besides serving as income generating high value crops. For instance, Cambodian farmers were able to generate a revenue of US$ 4,776/ha from Chinese kale cultivation, whereas Vietnamese farmers earned US$ 5,070/ha from water spinach cultivation (Genova et al., 2010). Vegetable production has led to 3–14 times higher profits per hectare than in rice farming in Cambodia and Vietnam, while profits per labor-day are double (Joosten et al., 2015). The leafy vegetables are repeat-cycle crops, and the average length of growing period is 7–8 weeks, though farmers often re-sow their field after harvest or use staggered sowing to extend the harvest period (Schreinemachers et al., 2017). Thus, leafy vegetables are an important source of income for smallholder farmers.

Leafy vegetables are also an important source of nutrients. The leafy vegetables including amaranth and Chinese kale supply vitamins (especially vitamins A, C, folate), minerals such as calcium, potassium, iron, phosphorus, zinc, copper and manganese and dietary fiber to the human diet (Makobo et al., 2010; Ebert et al., 2011; Fowler, 2011). Water spinach is rich in protein, calcium, pro-vitamin A and vitamin C (Westphal, 1994). In addition, water spinach is a key contributor of lutein/zeaxanthin (Pan et al., 2018). However, the production of leafy vegetables is highly seasonal. For instance, amaranth is the predominant leafy vegetable in the summer months in Taiwan. Although water spinach is also grown during the summer months, leafy brassicas are mostly produced during the cooler months (Wang and Ebert, 2012).

Production of leafy vegetables is constrained by abiotic and biotic factors. Typhoons, for example, which regularly hit Taiwan during summer months, are often associated with heavy rainfall of up to 3,000 mm and subsequent flooding. Such extreme weather conditions not only lead to heavy yield losses in leafy vegetables, but also escalates their prices in the market (Wang and Ebert, 2012). Prices may soar to 3–5 times the normal level (Lee and Yang, 1999), which reduce their consumption during the summer months. Biotic factors such as pests and diseases also adversely affect the productivity of leafy vegetables. The leaf webber, *Spoladea recurvalis* F. (Lepidoptera: Crambidae) causes significant yield losses on amaranth in Asia (Hsu and Srinivasan, 2012) and Africa (Smith et al., 2018). White rust of amaranth, caused by *Albugo bliti* (Biv.) Kuntze, is a serious problem during the hot and humid conditions in Taiwan as well as in Southeast Asia (Grubben and Van Sloten, 1981; National Research Council, 1984), which causes almost 100% infection, considerably reducing the commercial value of the crop. Spotted tortoise beetle, *Aspidomorpha miliaris* F. (Coleoptera: Chrysomelidae), Convolvulus hawk-moth, *Agrius convolvuli* L. (Lepidoptera: Sphingidae), sweet potato stem borer, *Omphisa anastomosalis* Guenée (Lepidoptera: Crambidae) and common armyworm, *Spodoptera litura* F. (Lepidoptera: Noctuidae) are the major pests of water spinach (Muniappan et al., 2012).

Most leafy vegetables can be successfully grown under protective structures year-round. The leafy vegetables grown under protective structures using plug seedlings have been demonstrated to be grown faster, and harvested earlier with fewer pest problems compared to conventional production using direct seeding (Lee and Yang, 1999). Production under protective structures not only increases the total annual crop yield per unit area, but also improves the quality, besides extending their production period (Nordey et al., 2017). In recent years, use of color shade nets was found to protect the crops from adverse environmental conditions, improve the quality of vegetables and maintain post-harvest quality for an extended period (Ilić et al., 2018). But there is no evidence yet on how these shades affect yield or nutritional content of leafy greens. Hence, the objective of the current study is to determine the effects of different protective structures on the horticultural and nutritional yield of amaranth and water spinach across the seasons in Taiwan.

MATERIALS AND METHODS

Field Trials

Location and Seasons

The study was conducted at the World Vegetable Center, Shanhua, Tainan, Taiwan (23°08′29″N, 120°19′15″E) at a mean elevation of 9 m above the sea level. The trials were conducted following a complete randomized block design (CRBD), with three blocks. Three field trials were conducted during fall season (Sept 23–Oct 28, 2020), winter season (Dec 09 2020–Feb 8 2021), and spring season (March 24–May 5, 2021).

Treatments and Data Collection

Three leafy vegetables, *viz*., amaranth (*Amaranthus tricolor*, cv. white amaranth and cv. red amaranth) and water spinach (*Ipomoea aquatica* cv. kangkong) were compared under white poly-net house (clear color pattern, allowing full sunlight spectrum), pink poly-net house (magenta color pattern, Blue: Green: Red: Far Red percentages B:G:R:FR = 40:32:60:127, and 80% density knitted shade net) and open field conditions. Nets were manufactured by SpectralX, LeBio International Technology Corp. Ltd, Tainan, Taiwan. Each poly-net house was 7 x 12 x 4-m (W:L:H). Hence, each production system was considered as a treatment, and four replications were maintained for each treatment. The seeds were obtained from Hsinyusen seed company (Yunlin County, Taiwan). The crops were sown on raised bed (9.2-m long and 1-m wide) in each replication and managed following the customary production practices, including surface irrigation at weekly intervals and manual weeding three times in the season. About 25 kg of organic fertilizer (compost) (Chung Rong Industrial Company, Tainan, Taiwan) was applied to each bed. The incidence of pests and diseases were recorded from each replication. Spider mite occurred in the fall and spring seasons, whereas aphid occurred in the winter. The spider mite damage was rated using a 0–5 scale (Nihoul et al., 1991) and aphid damage was rated using a 1–5 scale: 1 (<10 aphids), 2 (11–50 aphids), 3 (51–100 aphids), 4 (101–500 aphids) and 5 (>500 aphids). At harvest, the vegetables were sorted and graded as marketable and unmarketable, and the yield of each category was recorded. The climatic conditions (temperature and relative humidity) in each replication were recorded throughout the season. Besides horticultural yield, nutritional analysis including dry matter, anti-oxidant activity, iron, and carotenoids (violaxanthin, neoxanthin, lutein, α-Carotene and β-Carotene) was carried out in all the crops and treatments. At harvest, pooled plant samples were collected from each treatment and used for the nutritional analysis. Two biological replications were used for each crop, and the mean value for each nutritional compound was used to estimate the total nutritional yield from the unit area (one hectare).

Nutritional Analysis

Iron

AOAC method no. 975.03 was used for iron determination (AOAC, 1990). Briefly, 0.2 g of dried sample powder from each treatment was mixed with 5 ml of 36 N sulfuric acid in the digestion flask. The samples were then kept aside overnight. The elemental analysis was continued by heating the digestion tubes at 300°C for 2 h. The contents were then cooled to about 150°C, and 2–3 ml of 30% hydrogen peroxide (H2O2) was added. The tubes were placed in the digester at 300°C for 1h to make the mixture transparent. The mixture was then cooled to about 40°C, and diluted with 50 ml distilled water. The iron content was determined in each sample using inductively coupled plasma–optical emission spectrometry (ICP-OES) instrument (8000 ICP-OES, PerkinElmer, Waltham, MA, USA). The standards were also prepared to make the calibration curve.

Antioxidant Activity

Leafy vegetable samples were analyzed for antioxidant activity (AOA) by ARP method (Arnao et al., 2001). This method measures the capacity of different components to scavenge the ABTS radical cation as compared to the standard antioxidant Trolox (0–4 mM) in a dose response curve. 0.1 g of freeze-dried powder with 9.9 ml of methanol was added in a centrifuge tube. The mixture was shaken for 4 h at high speed, and centrifuged at 6,000 rpm for 10 min. The supernatant was transferred into vials and stored at −70°C until analyzed. The reaction mixture contained 10 ml of 20 mM ABTS / 50 mM sodium phosphate buffer (pH 7.5) with 0.5 ml of HRP stock solution and 90 ml of ethanol. The mixture was then centrifuged at 12,000 rpm for 5 min and the supernatant was collected. Twenty microliters of antioxidant sample with appropriate dilution in water or methanol was added to the 2 mL of the reaction medium. The decrease in absorbance, which was proportional to the ABTS quenched, was determined after 5 min by spectrophotometer (U-2001, HITACHI, Tokyo, Japan) at 730 nm. The AOA of a sample for the ARP assay was measured within the linear relationship of concentration vs. optical density decrease, and presented as Trolox equivalent (TE) in μmol/g vegetable sample (fresh weight basis).

Dry Matter

Dry matter was determined from the weight difference of 1.0 g of fine powder before and after placing in an oven (DN 63, Yamato, Tokyo, Japan) at 135°C for 2 h.

Carotenoids

The carotene content was determined using the high-performance liquid chromatography (HPLC) method (Rodriguez-Amaya and Kimura, 2004). Briefly, 0.1 g of freeze-dried powder was mixed thoroughly with 0.5 ml of distilled water and 4.5 ml of acetone in glass vial, and the mixture was shaken for 30 min. Two milliliters of supernatant were pipetted into 10 ml test tube, and then dried using N_2 gas at 36°C for 20 min. To the dried sample, 100 μl of tetrahydrofuran (THF) and 1,900 μl Methanol was added and mixed well. The solution was then filtered through a 0.22 μm membrane and the final solution of 2 ml was injected into HPLC vials by using glass syringes enclosed with 0.22 μm pore size, and 13 mm diameter syringe filter. Separation and identification of carotenoids was performed using a HPLC system (Waters 2695, Milford, MA, USA) equipped with an auto-sampler, a photodiode array detector (Waters 996) monitoring at wavelength between 210 and 700 nm. The static phase was a C 30 Column (YMC^{TM} Carotenoid 3.0 μm, 4.6 ×150 mm). The running conditions were set at 30°C using a gradient at 1.3 mL/min from 0 to 1% THF in methanol at 0–15 min, 1–25% THF in methanol at 15–25 min, 25–70% THF in methanol at 25–50 min, and the final 100% THF at 50–60 min. Identification of sample carotenoids was performed by comparing retention time and light absorption spectra (350–700 nm) of known standards. The peak areas were calibrated against known amounts of standards.

Estimating the Number of People That Can Receive Recommended Dietary iIntake of Iron and β-Carotene From Amaranth and Water Spinach Grown Under Different Production Conditions

Based on the nutritional yield per hectare, the number of people that can receive recommended dietary intake (RDI) of iron and β-Carotene from white amaranth, red amaranth and water spinach were estimated. We have attempted to pursue this perspective in the current study, since most of the existing studies attempt to understand the impact of agronomic practices on the yield but not on the nutrients. We chose only iron and β-carotene, although α-carotene was also a pro-vitamin A carotenoid. The revised bio-efficacy of α-carotene in a mixed diet is 1: 24 (Institute of Medicine, 2001), and hence it has been estimated to be 16.8–21.6 mg/day α-carotene that would fulfill the RDI for vitamin A of healthy adults. Since the α-carotene content in the amaranth and water spinach was comparatively lower than β-carotene, we did not include it in the estimation. Violaxanthin, neoxanthin and lutein are the epoxy carotenoids, which might be degraded by to the acidic conditions in the stomach (Asai et al., 2008; Britton et al., 2009; Maoka and Etoh, 2010), and hence we also did not include them in the estimation. The widely accepted

TABLE 1 | Analyses for marketable yield and nutritional content of white amaranth under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020-–2021.

Source	df	Yield		Dry matter		Fe		Violaxanthin		Neoxanthin		Lutein		α-Carotene		ß-Carotene		AOA	
		F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
Model	17	30.32	<0.0001	30.55	<0.0001	114.53	<0.0001	45.92	<0.0001	29.23	<0.0001	28.98	<0.0001	32.92	<0.0001	28.03	<0.0001	29.38	<0.0001
Season	2	88.46	<0.0001	90.84	<0.0001	225.10	<0.0001	134.51	<0.0001	76.20	<0.0001	75.72	<0.0001	45.23	<0.0001	70.23	<0.0001	81.03	<0.0001
Treatment	2	43.99	<0.0001	40.42	<0.0001	83.80	<0.0001	2.57	0.1100	67.31	<0.0001	67.49	<0.0001	155.55	<0.0001	78.09	<0.0001	56.92	<0.0001
Season ∗ Treatment	4	16.61	***<0.0001***	18.02	***<0.0001***	193.11	***<0.0001***	57.93	***<0.0001***	9.72	***0.0004***	9.35	***0.0005***	2.92	***0.0571***	6.40	***0.0033***	12.13	***0.0001***

*The bold values indicate the statistical significance for Season*Treatment.*

TABLE 2 | Analyses for marketable yield and nutritional content of red amaranth under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020–2021.

Source	df	Yield		Dry matter		Fe		Violaxanthin		Neoxanthin		Lutein		α-Carotene		ß-Carotene		AOA	
		F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
Model	17	10.20	<0.0001	10.23	<0.0001	13.10	<0.0001	10.71	<0.0001	10.53	<0.0001	10.69	<0.0001	13.64	<0.0001	10.45	<0.0001	10.22	<0.0001
Season	2	77.92	<0.0001	72.59	<0.0001	111.77	<0.0001	89.24	<0.0001	66.97	<0.0001	63.88	<0.0001	42.74	<0.0001	66.78	<0.0001	73.10	<0.0001
Treatment	2	7.34	0.0060	12.56	*0.0006*	8.89	0.0028	1.35	0.2890	20.26	*<0.0001*	23.63	*<0.0001*	61.58	*<0.0001*	19.05	*<0.0001*	12.09	*0.0007*
Season ∗ Treatment	4	3.24	***0.0419***	2.41	0.0951	11.46	***0.0002***	5.75	***0.0052***	1.88	0.1662	1.63	0.2186	2.26	0.1107	1.79	0.1842	2.48	0.0886

*The bold values indicate the statistical significance for Season*Treatment.*

TABLE 3 | Analyses for marketable yield and nutritional content of water spinach under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020–2021.

Source	df	Yield		Dry matter		Fe		Violaxanthin		Neoxanthin		Lutein		α-Carotene		ß-Carotene		AOA	
		F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
Model	17	12.24	<0.0001	13.01	<0.0001	33.67	<0.0001	14.32	<0.0001	19.01	<0.0001	17.86	<0.0001	31.29	<0.0001	17.04	<0.0001	18.64	<0.0001
Season	2	35.02	<0.0001	38.30	<0.0001	46.05	<0.0001	32.33	<0.0001	26.38	0.0002	27.34	0.0001	17.95	0.0007	31.09	<0.0001	46.14	<0.0001
Treatment	2	12.40	0.0005	6.13	0.0099	140.96	<0.0001	38.34	0.1100	96.26	<0.0001	83.34	<0.0001	218.97	<0.0001	63.79	<0.0001	20.42	<0.0001
Season ∗Treatment	4	22.13	***<0.0001***	25.18	***<0.0001***	35.11	***<0.0001***	18.19	***<0.0001***	12.38	***<0.0001***	13.22	***0.0005***	6.36	***0.0026***	16.90	***<0.0001***	34.88	***<0.0001***

*The bold values indicate the statistical significance for Season*Treatment.*

RDI value of 8 mg/day (for men 19+ and women 51+) and 18 mg/day (for women 19–50) for iron was used in the estimation. The revised bio-efficacy of β-carotene in a mixed diet is 1:12, whereas it is 1:24 for other pro-vitamin A carotenoids (Institute of Medicine, 2001). The widely accepted recommended dietary allowance (RDA) for vitamin A is 900 μg RAE for men (14+) and 700 μg RAE for women (14+), except the pregnant and lactating women. Thus, it has been estimated to be 10.8 mg/day β-carotene that would fulfill the RDA for vitamin A (900 μg) for healthy men and 8.4 mg/day for healthy women (700 μg) (Böhm et al., 2020). Hence, these RDI/RDA values for iron and β-carotene were used to estimate the number of people that can receive these nutrients from the crop harvests in the current study.

Data Analysis

The data was analyzed using ANOVA with the procedure Proc GLM of SAS version 9.4 (SAS Institute, Cary, NC, USA). The significant differences were identified and means were separated by Tukey's HSD test (differences were considered significant at $\alpha = 0.05$). Data on spider mite infestation did not follow normal distribution (even with data transformation). Therefore, a non-parametrical analysis was conducted. Each season was independently analyzed/crop, using the NPAR1WAY Procedure in SAS. Distribution of Wilcoxon Scores for spider mite percentages was analyzed with a Kruskal-Wallis Test. Later, a pairwise Two-Sided Multiple Comparison Analysis Dwass-Steel-Critchlow-Fligner Method was conducted to get differences between specific treatments per season/crop. For aphid infestation, categorical data was analyzed using the CATMOD procedure in SAS and the analysis of variance and the Analysis of Weighted Least Squares Estimates was done using a Chi-Square test for the treatment parameter. Data on diseases was transformed ASIN[SQRT(x)] for normality and UNIVARIATE Procedure was conducted to confirmed data had a fitted normal distribution following a Shapiro-Wilk test for normality. Combined analysis was conducted to evaluate differences among seasons in each crop. Non-transformed data is presented in the results section.

RESULTS

Marketable Yield of Amaranth and Water Spinach

Interaction effects (Treatment*Season) showed significant difference for marketable yield (**Tables 1–3**). White amaranth yield was significantly higher under pink poly-net house (19.70 t/ha) in the fall season, followed by open field (19.30 t/ha) and the white poly-net house (17.88 t/ha), which were on par with each other (**Figure 1A**). The yield was generally lower in the winter season compared to the fall season. In winter, both the pink poly-net house (15.10 t/ha) and the white poly-net house (15.30 t/ha) recorded similar yield, but it was significantly lower in the open field conditions (5.70 t/ha). Among the three seasons, spring recorded the lowest marketable yield in all the three production conditions. Like winter, both the pink poly-net house (9.75 t/ha) and the white poly-net house (9.83 t/ha) recorded similar yield, but nothing was harvested from the open field conditions.

The yield of red amaranth was also significantly higher under pink poly-net house (16.95 t/ha) in the fall season, followed by open field (13.50 t/ha) and the white poly-net house (13.45 t/ha), which were on par with each other (**Figure 1B**). Unlike white amaranth, the yield of red amaranth was slightly higher in the winter season compared to the fall season. In winter also, the poly-net houses recorded significantly higher yield (up to 18.17 t/ha), followed by the open field conditions (16.90 t/ha). Spring recorded the lowest marketable yield in all the three production conditions. Both the pink poly-net house (7.68 t/ha) and the white poly-net house (8.37 t/ha) recorded similar yield, but nothing was harvested from the open field conditions.

In the fall season, both open field (17.84 t/ha) and the white poly-net house (16.25 t/ha) recorded higher yield of water spinach than the pink poly-net house (14.80 t/ha) (**Figure 1C**). However, in the winter, this crop yielded significantly higher only under the white poly-net house conditions (19.70 t/ha), followed by pink poly-net house (16.07 t/ha), but the harvest from the open field was much lower (6.93 t/ha). In contrary to the amaranth crops, water spinach yielded significantly higher during the spring season. The yield was similar in all the three production conditions and ranged 20.55–20.88 t/ha.

Pests and Diseases

Spider mite (*Tetranychus* spp.) appeared to be the predominant pest on all the three crops in the poly-net houses, irrespective of their color in the fall and spring seasons (**Figure 2**). However, open-field water spinach was also infested by the mites in the fall season. Aphids were recorded as the major pest in the winter crop. About 77.5% of the red amaranth (Chi-square = 431.38, $P < 0.0001$) and 100% of the white amaranth (Chi-square = 88.40, $P < 0.0001$) in white poly-net house had reached the damage score of 5, whereas it was 75% in both red amaranth and white amaranth plants in the pink poly-net house condition (**Figure 3**). Although 30% of the red amaranth in open field condition were mildly (1–2 score) infested by aphids, the white amaranth was completely free from aphid infestation. Similarly, a vast majority of water spinach plants (72.5%) were free from aphids (Chi-square = 352.16, $P < 0.0001$), but about 87.5% of plants in the white poly-net house and about 75% of plants in the pink poly-net house were infested by aphids. There was no disease infecting the water spinach. However, white and red amaranths were infected by *Pythium* and *Rhizoctonia*. Interaction effects (Treatment*Season) showed significant difference for disease incidence on white amaranth, but not on red amaranth (**Table 4**). The disease was comparatively severe on white amaranth than on red amaranth (**Table 5**), but more or less similar in all the three production conditions, with the maximum incidence of 7.55% during the fall season.

Nutritional Content

Interaction effects (Treatment*Season) showed significant difference for all the nutritional parameters of white amaranth (**Table 1**). The dry matter content was corresponding to the yield of the crop under different production conditions (**Table 6**).

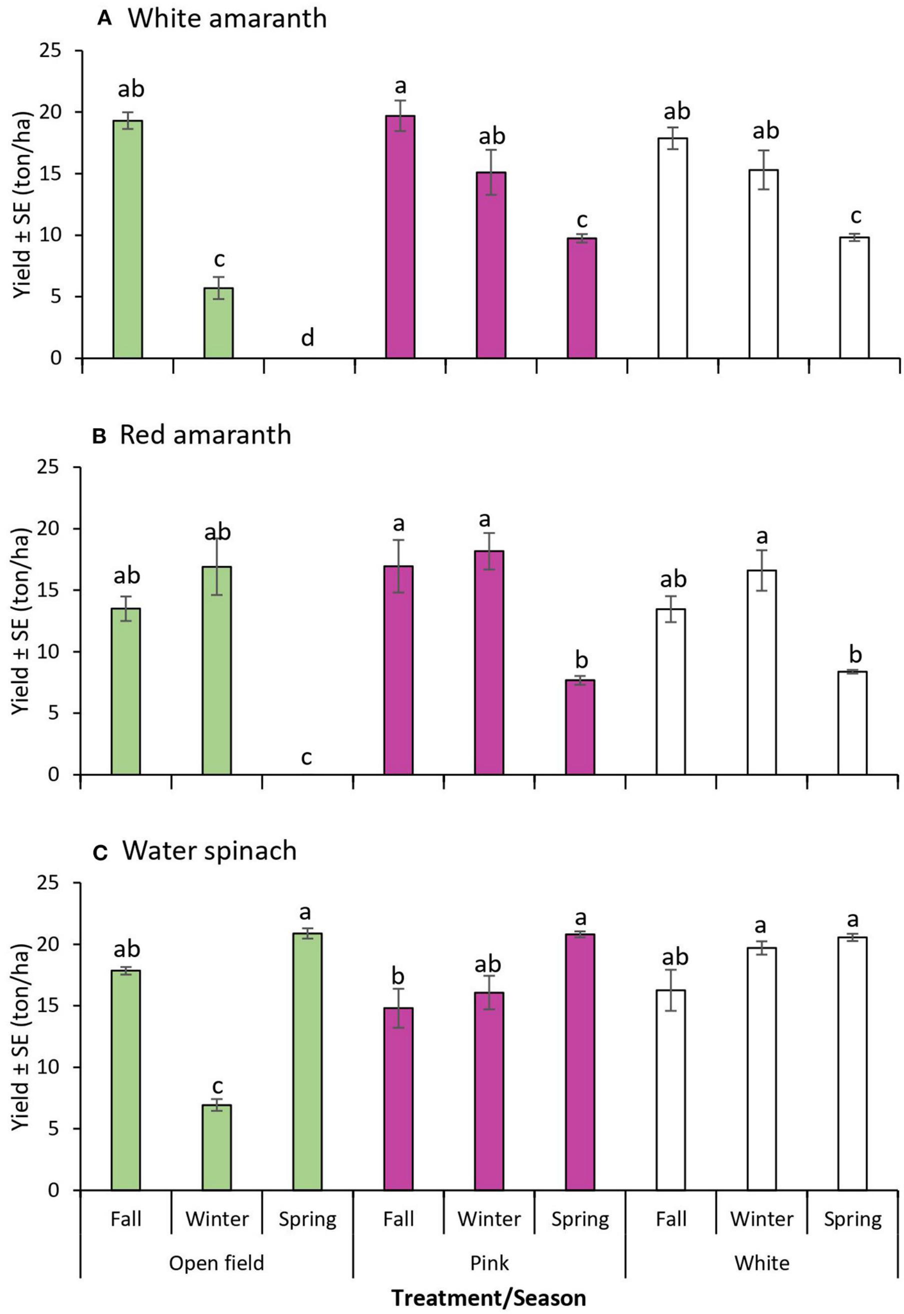

FIGURE 1 | Yield ± SE (ton/ha) of leafy vegetables grown under different production conditions. **(A)** White amaranth, **(B)** Red amaranth, **(C)** Water spinach. Means represent average of four replications in each treatment/season. Means followed by the same letter(s) in a crop are not significantly different ($p < 0.05$).

The iron content was significantly higher during the fall season in the white amaranth harvested from the open field (881.67 g/ha), followed by pink poly-net house (336.48 g/ha) and white poly-net house (234.50 g/ha). However, the open field (260.39 g/ha) and the pink poly-net house (257.91 g/ha) supplied more iron than the white poly-net house (200.72 g/ha) in the winter season. Since no crop was harvested from the open field during the spring season, there was no iron supply from the open field condition. However, both the poly-net houses yielded similar amount of iron (128.89–166.53 g/ha) during the spring. Although open field production of white amaranth supplied better violaxanthin, neoxanthin and lutein in the fall season, it fell behind the poly-net houses in the winter crop. Supply of these compounds from the pink poly-net house was

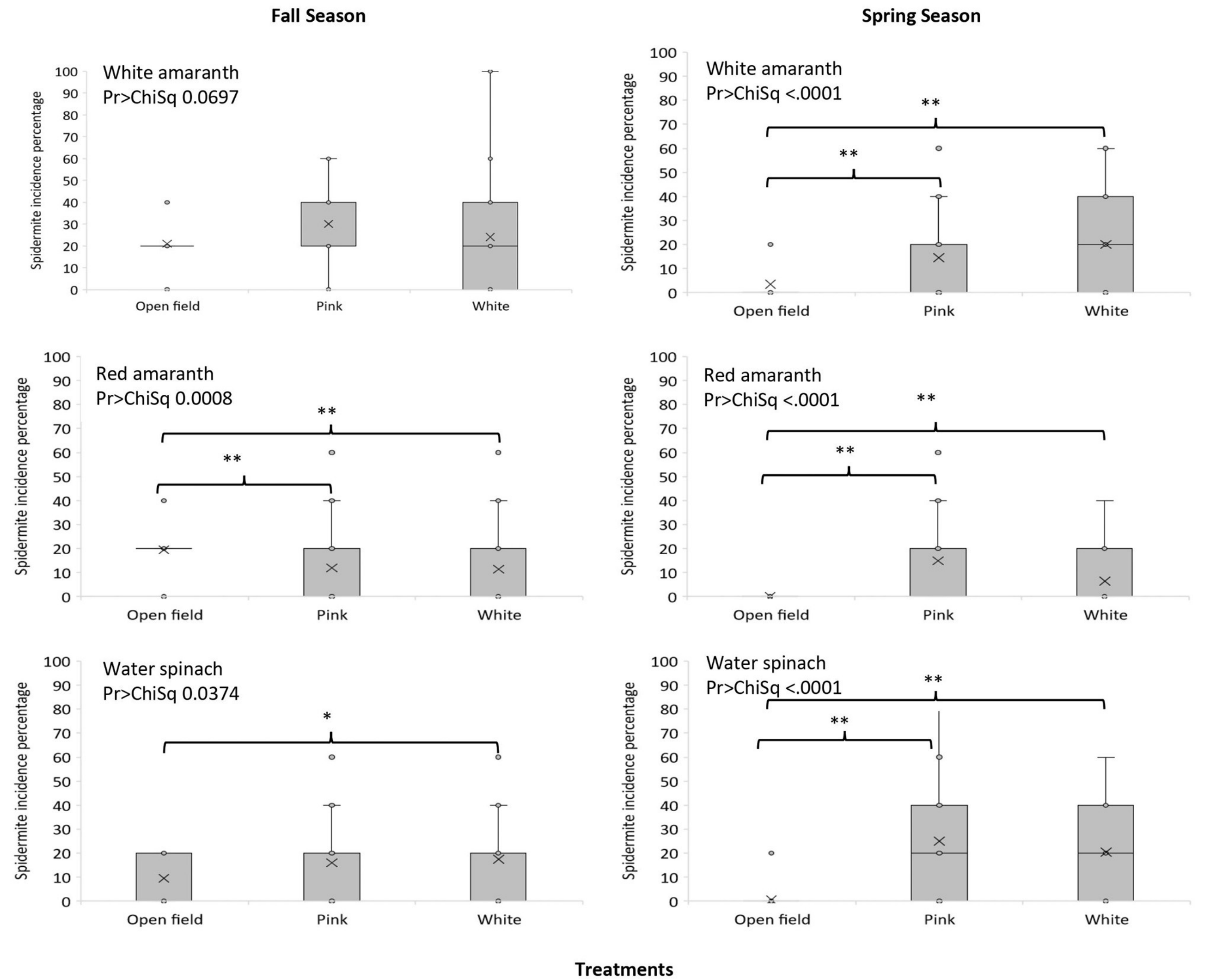

FIGURE 2 | Mean (±SD) incidence of spider mite (*Tetranychus* spp.) on leafy vegetables grown under different production conditions. **(A)** White amaranth, **(B)** Red amaranth, **(C)** Water spinach. Means represent average of four replications in each treatment/season. *significant; **highly significant.

significantly higher than from the white poly-net house in both fall and winter crops. Similarly, both the poly-net houses provided significantly higher amount of α-Carotene (18.90–47.18 g/ha) and β-Carotene (362.32–732.06 g/ha) than the open field crops (0–16.06 g/ha and 0–556.29 g/ha) during these seasons. Consistently, better anti-oxidant activity was recorded for both the poly-net houses (91.68–190.24 mole/ha) than the open field (0–165.43 mole/ha) in the fall and winter seasons. However, all the nutritional parameters were significantly higher in both the poly-net houses than in the open field during the spring season.

Interaction effects (Treatment*Season) showed significant difference only for iron and violaxanthin content of red amaranth (**Table 2**). The iron content was significantly higher in red amaranth produced from open field (645.42 g/ha), compared to the white poly-net house (352.14 g/ha) during the fall season (**Table 7**). However, it was intermediate in the crop produced from the pink poly-net house (574.04 g/ha). Similar trend was also recorded in the winter season. The violaxanthin supply was almost on par in all the treatments in both fall (798.47–954.43 g/ha) and winter (985.47–1178.38 g/ha) seasons. In red amaranth as well, there was no supply of iron and violaxanthin from open field production during spring, but both the poly-net houses provided equal amounts (432.31–496.74 g/ha). In addition, dry matter content, carotenoids and anti-oxidant activity of red amaranth only responded to treatment effect (**Table 8**). Moreover, pink poly-net house recorded significantly higher amount of all the other nutritional compounds (**Table 8**). White poly-net house also recorded on par values for most of the parameters, except for neoxanthin, lutein and α-Carotene. All these nutritional compounds were significantly lower in red amaranth harvested from the open field, compared to the poly-net houses.

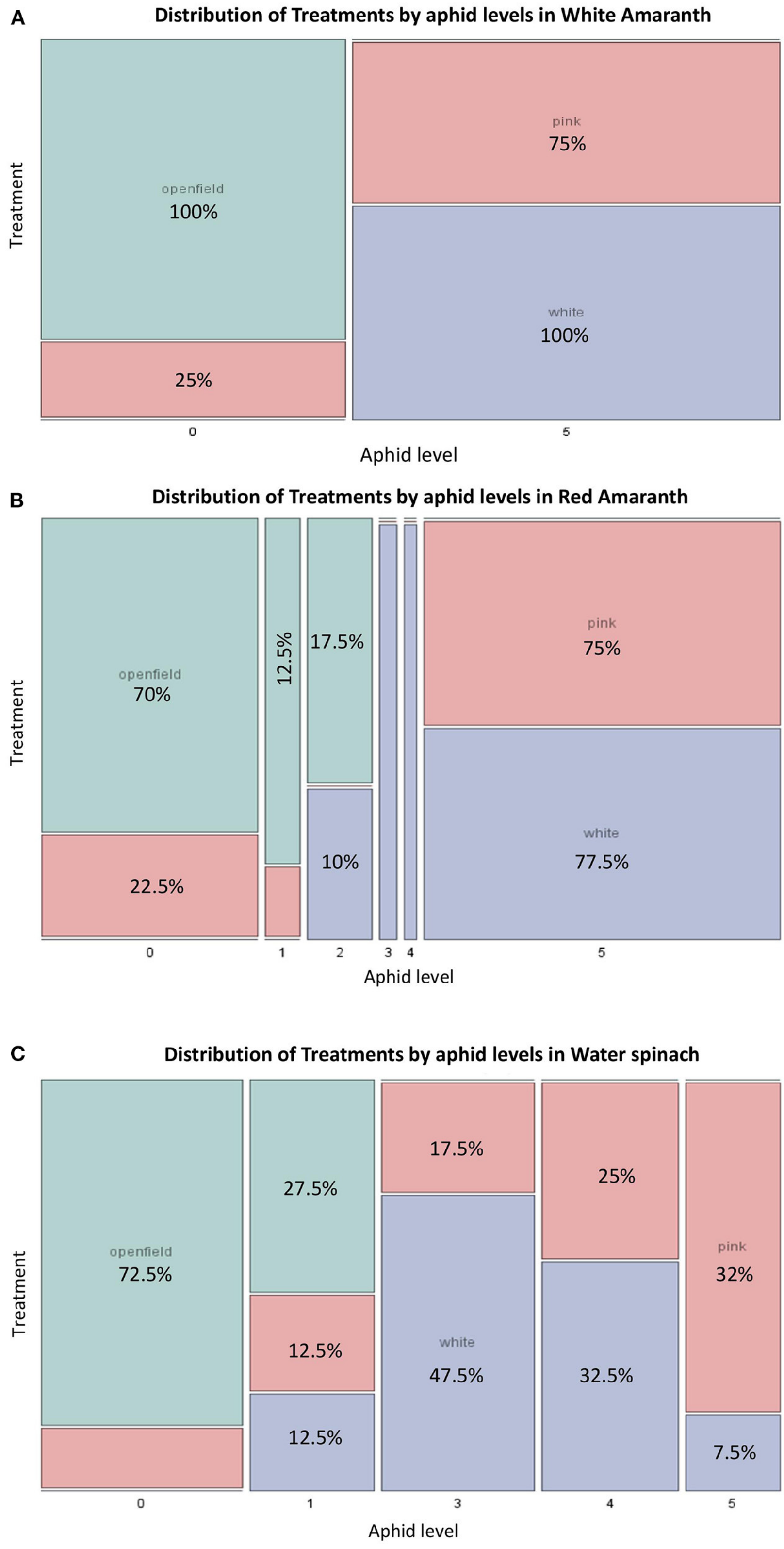

FIGURE 3 | Mean incidence of aphids on leafy vegetables grown under different production conditions. **(A)** White amaranth, **(B)** Red amaranth, **(C)** Water spinach. Means represent average of four replications in each treatment/season.

TABLE 4 | Analyses for disease incidence on white amaranth and red amaranth under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020–2021.

Source	df	White Amaranth		Red Amaranth	
		F	Pr > F	F	Pr > F
Model	17	43.12	<0.0001	1.02	0.48
Season	2	21.48	0.0004	8.58	0.0082
Treatment	2	97.44	<0.0001	3.42	0.0578
Season*Treatment	4	49.09	<0.0001	1.20	0.3504

TABLE 5 | Disease incidence (%) on white amaranth and red amaranth under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020–2021.

Season	Treatment	White Amaranth		Red Amaranth	
		N	Disease percentage ± SE	N	Disease percentage ± SE
Fall	Open field	4	6.37 ± 1.23 ab	4	1.80 ± 0.22
	Pink	4	7.55 ± 1.14 a	4	1.94 ± 0.15
	White	4	7.55 ± 1.14 a	4	2.24 ± 1.04
Winter	Open field	4	0 e	4	0.00
	Pink	4	3.96 ± 0.63 cd	4	2.37 ± 0.90
	White	4	4.63 ± 0.53 bc	4	1.32 ± 0.79
Spring	Open field	4	2.86 ± 0.24 d	4	0.83 ± 0.10
	Pink	4	3.04 ± 0.13 d	4	1.92 ± 0.07
	White	4	3.40 ± 0.05 cd	4	1.84 ± 0.26

Means followed by the same letter(s) in a column are not significantly different ($p < 0.05$).

Interaction effects (Treatment*Season) showed significant difference for all the nutritional parameters of water spinach (**Table 3**). The dry matter content was significantly higher in the water spinach harvested from the open field during fall season (2,128.6 kg/ha), but it was the lowest in the winter season (826.2 kg/ha) (**Table 9**). In spring, the dry matter content was significantly higher in the plants harvested from pink poly-net house (2,520.7 kg/ha), followed by the open field (2,490.6 kg/ha). The iron content was significantly higher during the fall season in the water spinach harvested from the open field (361.7 g/ha), followed by pink poly-net house (298.4 g/ha), but only the pink poly-net house supplied more iron (323.9 g/ha) than the other two production conditions (140.4–168.3 g/ha) in the winter season. Both the open field condition (423.2 g/ha) and the pink poly-net house (419.3 g/ha) yielded significantly higher amount of iron during the spring. Consistently higher number of carotenoids were obtained from the water spinach harvested under the pink poly-net house in all the seasons, and it was significantly higher during the spring than the other seasons. Significantly higher anti-oxidant activity was recorded for the open field in the fall (336.3 mole/ha) and spring (393.4 mole/ha) seasons, which was on par with the pink poly-net house (344.5 mole/ha) in the spring.

TABLE 6 | Dry matter content, iron, carotenoids and anti-oxidant activity of white amaranth under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020–2021.

Season	Treatment	Dry matter (kg/ha)	Fe (g/ha)	Violaxanthin (g/ha)	Neoxanthin (g/ha)	Lutein (g/ha)	α-Carotene (g/ha)	β-Carotene (g/ha)	AOA, (mole/ha)
Fall	Open field	2,455.48 ± 85.66 a	881.67 ± 30.76 a	533.26 ± 18.60 a	222.04 ± 7.75 ab	831.75 ± 29.02 ab	16.06 ± 0.56 d	556.29 ± 19.40 b	165.43 ± 5.77 ab
	Pink	2,393.01 ± 150.83 a	336.48 ± 21.21 b	342.67 ± 21.60 b	280.90 ± 17.70 a	1,049.44 ± 66.14 a	47.18 ± 2.97 a	732.06 ± 46.14 a	190.24 ± 11.99 a
	White	2,246.90 ± 110.06 a	234.50 ± 11.59 cd	310.09 ± 15.33 bc	234.98 ± 11.61 ab	899.50 ± 44.46 ab	34.39 ± 1.70 b	700.61 ± 34.63 ab	166.80 ± 8.25 ab
Winter	Open field	725.19 ± 114.50 c	260.39 ± 41.11 bc	157.49 ± 24.87 d	65.58 ± 10.35 de	245.65 ± 38.79 de	4.74 ± 0.75 e	164.29 ± 25.94 d	48.85 ± 7.71 d
	Pink	1,834.25 ± 222.77 a	257.91 ± 31.32 bc	262.66 ± 31.90 bc	215.31 ± 26.15 ab	804.39 ± 97.70 ab	36.16 ± 4.39 b	561.13 ± 68.15 ab	145.82 ± 17.71 ab
	White	1,923.22 ± 198.61 a	200.72 ± 20.73 cde	265.42 ± 27.41 c	201.13 ± 20.77 b	769.92 ± 79.51 b	29.44 ± 3.04 bc	599.69 ± 61.93 ab	142.77 ± 14.73 b
Spring	Open field	0 c	0 e	0 e	0 e	0 e	0 e	0 d	0 d
	Pink	1,184.36 ± 41.64 b	166.53 ± 5.85 de	169.60 ± 5.96 d	139.03 ± 4.89 c	519.39 ± 18.26 c	23.35 ± 0.82 cd	362.32 ± 12.74 c	94.15 ± 3.31 c
	White	1,235.01 ± 37.14 b	128.89 ± 3.88 de	170.44 ± 5.13 d	129.16 ± 3.88 cd	494.41 ± 14.87 cd	18.90 ± 0.57 d	385.09 ± 11.58 c	91.68 ± 2.76 c

Means followed by the same letter(s) in a column are not significantly different ($p < 0.05$).

Number of People That Can Receive Recommended Dietary Intake of Iron and β-Carotene

Based on the widely accepted RDI of iron, open field produced white amaranth from a hectare was estimated to supply iron to 68,149-80,896 more men (19+ years old) and women (51+ years old) than the crop produced under poly-net houses during the fall season (**Table 10**). In the winter, the white amaranth produced from the open field conditions as well as the pink poly-net houses was estimated to supply iron to almost equal number (>7,000 people) of men and women (51+). In addition, water spinach produced under the pink poly-net houses during winter was estimated to provide iron to 19,450–22,939 more men (19+) and women (51+) than the other production conditions. In the spring, the white and red amaranths produced under poly net-houses supplied the iron, and the pink poly-net house was estimated to supply to 2,000–5,000 more men and women than the white poly-net house. In case of women (19–50 years), the same trend in the results was also recorded for all the crops, seasons and production conditions. β-Carotene supply to both men (14+) and women (14+) was consistently higher in all the crops produced under the pink ploy-net houses in all the seasons, except for the white amaranths produced under white poly-net house during the winter. The maximum supply from the red amaranth produced under pink poly-net houses reached a value of 64,788 more men and 83,298 more women than the other production conditions in all the seasons (**Table 10**). Thus, the pink poly-net house was found to be the most suitable production condition for supplying β-Carotene to a maximum number of people.

DISCUSSION

In this study, we investigated the effect of different colored poly-net house compared to open field conditions in terms of yield, dry matter content, iron, carotenoids and anti-oxidant activity for three leafy vegetables, *viz*., white amaranth, red amaranth, and water spinach, over three seasons (fall, winter and spring). In addition, the overall effect of pests and diseases was also evaluated under the above-mentioned conditions. Finally, the number of people who could receive selected micro-nutrients from the crops harvested under different production conditions was estimated based on the RDI values. This is quite important to understand if sufficient quantity of nutrient-dense vegetables can be grown in a relatively small area, considering the context of fast shrinking farmlands. To our knowledge, there are not many scientific information available on this perspective.

Generally, the amaranth yield was higher in the fall season, which could be due to the higher prevailing temperature during this season, ranging between 25.7 and 27.2°C (**Table 11**). An increased temperature (from 28 to 32°C) was found to promote both root and leaf growth in edible amaranth (*A. tricolor* cv. White leaf) in Taiwan (Hwang et al., 2018). Amaranth is a C4 plant, and an earlier study had demonstrated that photorespiration losses in C4 plants were limited and hence C4 plants had higher net photosynthetic rates at higher temperatures compared to C3 plants (Long, 1999). Hence, the higher temperature during the fall season was found to have a positive effect on the growth of this C4 crop. Besides temperature, the light conditions inside the poly-net houses were believed to have provided the optimum microclimate for growth and development of amaranth crops. For instance, the blue irradiance (400–500 nm) inside the pink net house was about 40%, whereas it was about 25% in the white poly-net house and open field conditions. It was already demonstrated that the blue (400–450 nm) polyethylene shade condition produced taller plant height, a greater number of leaves, biomass yield and bioactive compounds in red amaranth, compared with other conditions of films (Khandaker et al., 2010). This might be due to the fact that some plant species are more sensitive to blue light (Casal, 1994). Thus, the combination of higher temperature and blue light in the poly-net houses could have contributed for the better yield. Although both white and red amaranths are C4 plants, the yield of red amaranth was slightly higher in the winter season compared to the fall season, whereas it was not

TABLE 7 | Iron and violaxanthin content of red amaranth under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020–2021.

Season	Treatment	Fe (g/ha)	Violaxanthin (g/ha)
Fall	Open field	645.42 ± 47.61 ab	941.31 ± 69.44 a
	Pink	574.04 ± 72.43 abc	954.43 ± 120.43 a
	White	352.14 ± 27.55 cd	798.47 ± 62.48 ab
Winter	Open field	807.97 ± 109.96 a	1,178.38 ± 160.37 a
	Pink	615.25 ± 50.39 abc	1,022.94 ± 83.79 a
	White	434.61 ± 43.02 bcd	985.47 ± 97.55 a
Spring	Open field	0 e	0 c
	Pink	260.01 ±11.89 d	432.31 ± 19.78 b
	White	219.07 ± 3.84 d	496.74 ± 8.72 b

Means followed by the same letter(s) in a column are not significantly different ($p < 0.05$).

TABLE 8 | Dry matter content, carotenoids and anti-oxidant activity of red amaranth under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020–2021.

Treatment	Dry matter (kg/ha)	Neoxanthin (g/ha)	Lutein (g/ha)	α-Carotene (g/ha)	β-Carotene (g/ha)	AOA (mole/ha)
Open field	1,461.99 ± 376.39 b	484.94 ± 124.85 c	1,380.93 ± 355.52 c	54.95 ± 14.15 c	723.69 ± 186.31 b	134.09 ± 34.52 b
Pink	2,630.93 ± 307.10 a	1,015.99 ± 118.59 a	3,085.79 ± 360.19 a	241.31 ± 28.17 a	1,487.38 ± 173.62 a	238.84 ± 27.88 a
White	2,290.67 ± 211.18 a	815.31 ± 75.17 b	2,468.60 ± 227.59 b	166.28 ± 15.33 b	1,243.68 ±114.66 a	208.33 ± 19.21 a

Means followed by the same letter(s) in a column are not significantly different ($p < 0.05$).

TABLE 9 | Dry matter content, iron, carotenoids and anti-oxidant activity of water spinach under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020–2021.

Season	Treatment	Dry matter (kg/ha)	Fe (g/ha)	Violaxanthin (g/ha)	Neoxanthin (g/ha)	Lutein (g/ha)	α-Carotene (g/ha)	β-Carotene (g/ha)	AOA (mole/ha)
Fall	Open field	2,128.6 ± 37.3 abc	361.7 ± 6.3 ab	502.2 ± 8.8 bc	402.3 ± 7.0 d	1,142.0 ± 20.0 c	17.1 ± 0.3 de	562.3 ± 9.8 bc	336.3 ± 5.9 ab
	Pink	1,793.5 ± 192.2 cd	298.4 ± 32.0 b	541.1 ± 58.0 bc	581.2 ± 62.3 bc	1,559.8 ± 167.1 b	49.0 ± 5.2 b	660.6 ± 70.8 bc	245.1 ± 26.3 c
	White	1,594.3 ± 163.7 d	138.8 ± 14.2 c	409.9 ± 42.1 c	379.3 ± 38.9 d	1,055.0 ± 108.3 c	28.8 ± 3.0 cd	436.5 ± 44.8 d	195.2 ± 20.0 cd
Winter	Open field	826.2 ± 56.9 e	140.4 ± 9.7 c	194.9 ± 13.4 d	156.1 ± 10.7 e	443.2 ± 30.5 d	6.7 ± 0.5 e	218.2 ± 15.0 e	130.5 ± 9.0 d
	Pink	1,947.0 ± 166.9 bcd	323.9 ± 27.8 b	587.4 ± 50.4 b	631.0 ± 54.1 b	1,693.3 ± 145.2 b	53.2 ± 4.6 b	717.2 ± 61.5 b	266.1 ± 22.8 bc
	White	1,932.7 ± 53.4 cd	168.3 ± 4.6 c	497.0 ± 13.7 bc	459.8 ± 12.7 cd	1,279.0 ± 35.4 bc	34.9 ± 1.0 c	529.2 ± 14.6 cd	236.6 ± 6.5 c
Spring	Open field	2,490.6 ± 50.5 ab	423.2 ± 8.6 a	587.6 ± 11.9 b	470.7 ± 9.5 bcd	1,336.1 ± 27.1 bc	20.1 ± 0.4 d	657.9 ± 13.3 bc	393.4 ± 8.0 a
	Pink	2,520.7 ± 30.1 a	419.3 ± 5.0 a	760.5 ± 9.1 a	816.9 ± 9.7 a	2,192.1 ± 26.2 a	68.9 ± 0.8 a	928.4 ± 11.1 a	344.5 ± 4.1 a
	White	2,016.1 ± 28.5 bcd	175.6 ± 2.5 c	518.4 ± 7.3 bc	479.6 ± 6.8 bc	1,334.2 ± 18.8 bc	36.4 ± 0.5 c	552.0 ± 7.8 bcd	246.8 ± 3.5 c

Means followed by the same letter(s) in a column are not significantly different ($p < 0.05$).

TABLE 10 | Number of people that can receive recommended daily intake of iron and β-Carotene from white amaranth, red amaranth and water spinach grown under colored poly-net house, white poly-net house, and open field conditions in Taiwan during 2020–2021.

Season	Treatment	Iron						β-Carotene					
		White amaranth		Red amaranth		Water spinach		White amaranth		Red amaranth		Water spinach	
		Men (>19) & Women (>51)	Women (19–50)	Men (>19) & Women (>51)	Women (19–50)	Men (>19) & Women (>51)	Women (19–50)	Men (>14)	Women (>14)	Men (>14)	Women (>14)	Men (>14)	Women (>14)
Fall	Open field	110,209	48,982	80,678	35,857	45,218	20,097	51,508	66,225	103,031	132,469	52,064	66,939
	Pink	42,060	18,693	71,755	31,891	37,298	16,577	67,783	87,150	167,819	215,767	61,169	78,645
	White	29,313	13,028	44,018	19,563	17,355	7,713	64,871	83,406	120,948	155,505	40,416	51,963
Winter	Open field	32,549	14,466	100,996	44,887	17,551	7,801	15,212	19,558	128,981	165,832	20,208	25,982
	Pink	32,239	14,328	76,906	34,181	40,490	17,996	51,956	66,801	179,865	231,255	66,404	85,376
	White	25,090	11,151	54,326	24,145	21,040	9,351	55,527	71,392	149,275	191,925	48,997	62,996
Spring	Open field	0	0	0	0	52,906	23,514	0	0	0	0	60,917	78,321
	Pink	20,816	9,252	32,501	14,445	52,419	23,297	33,548	43,133	76,014	97,732	85,967	110,529
	White	16,111	7,161	27,384	12,171	21,948	9,754	35,656	45,844	75,244	96,743	51,111	65,714

the case for white amaranth. Hence, besides temperature and light conditions, other factors may also contribute to these yield differences across the seasons. For instance, the photosynthetic pigments of higher plants include chlorophyll and carotenoids. The carotenoids content of the red amaranth were higher than the white amaranth, and most of the carotenoids in the white amaranth were lower in the winter season than in the fall. Hence, future studies should elucidate the role of photosynthetic pigments on yield under different production systems across the growing seasons. In addition, comparatively lower yield in the spring season, despite the warmer temperature could have been attributed to the higher spider mite infestation during this season. The growth and performance of water spinach was relatively better in the poly-net houses in all the seasons compared to the open field conditions, in which the lowest yield was recorded in the winter. Water spinach has originated from tropical regions, with high tolerance to heat and wet (Liou, 1981). The optimal growth conditions are temperatures between 20 and 27°C and the humidity of above 75% (Pinker et al., 2007). Since the winter temperature was quite low (**Table 11**), especially in the open field conditions, the yield might have declined significantly.

It is not surprising to record the spider mite infestation during the fall and spring season, in which the temperature is relatively warmer than the cold winter in Taiwan (**Table 11**). Most spider mites prefer warm and dry conditions. Although spider mite populations are found on crops in winter months in Taiwan (Ho, 2000), we did not find mite infestation on both the amaranth varieties and water spinach during the winter season. This is also due to the fact that aphids emerged as a major pest during the winter months and hence they outcompete spider mites. Aphids have been recorded as a serious pest of amaranth (Ebert et al., 2011). In fact, aphids occurred highly inside the poly-net houses. Several earlier studies have documented the aphid outbreaks inside the net house conditions (Talekar et al., 2003; Majumdar and Powell, 2011). A warm weather inside the poly-net houses during the winter months and the absence of natural enemies inside the protective structures could have led to the proliferation of aphid infestation in the current study. The major entry point for aphids and mites is through the nylon net. Most net houses were built with 40-mesh netting. The 40-mesh or coarser mesh nets failed to exclude the thrips, whitefly and aphid in several countries (Talekar et al., 2003; Harmanto, 2006). The poly-net houses used in the current study have been built with 32-mesh netting only. Hence, they were not able to prevent the entry of aphids and spider mites. The most promising way to prevent the entry of small-sized insects and mites in net houses is to use nets with finer mesh. Nets with 50-mesh or 60-mesh size to exclude thrips, whitefly, and aphids have been suggested (Polston and Lapidot, 2007; Shahak et al., 2008; Palada and Wu, 2009). However, finer mesh size reduces the ventilation rate and increases the relative humidity inside the net houses (Harmanto et al., 2006), which could favor the incidence of diseases, although it was not the case in the current study. It is interesting to note that the crop yields in both the poly-net houses were similar or higher than the open field production conditions despite the higher incidences of spider mite or aphids inside the poly-net houses. Hence, proper control options to manage aphids and spider mite on leafy vegetables in poly-net house conditions should be considered so that the crop productivity can be further increased.

Among the three production conditions compared in the current study, most of the carotenoids consistently occurred in higher quantities in the white amaranth crop produced under pink poly-net houses. Neoxanthin, lutein, and β-carotene were slightly higher in the red amaranth grown under the poly-net houses. Similarly, α-carotene was also higher in the amaranth crops grown under the poly-net houses. A recent study conducted under open field conditions in the same location documented higher amounts of violaxanthin, neoxanthin and lutein (Nordey et al., 2021) than the white amaranth in the current study, but the α- and β-carotenes were similar in both the studies. However, all the carotenoids in the previous study were lower than the quantities in the red amaranth in our study. It should be noted that the previous study involved amaranth accessions from different species (*Amaranthus hypochondriacus*, *A. cruentus, A. dubius* and *A. blitum*), whereas the varieties in the current study belonged to *A. tricolor*. In line with this, other papers on amaranth studying the carotenoid profiles corroborated our results, where higher carotenoids in the red amaranth have been observed compared to other genotypes (Khanam and Oba, 2013; Sarker and Oba, 2020). Hence, the quantity of carotenoids not only differed among the species of amaranth, but also varied among the varieties within a species. In addition, these variations could be altered by the production conditions, as we observed in the case of red amaranth under poly-net house conditions compared to the open field conditions. Hence, protected cultivation conditions not only lead to more marketable yields but also result in higher quantities of health promoting nutrients.

The quantities of the carotenoids such as lutein and β-carotene observed in water spinach in the current study were similar to the earlier findings (Khoo et al., 2011; Chandra-Hioe et al., 2017), and the α-carotene was 10–25-fold higher than an earlier report (Khoo et al., 2011). As observed in the case of amaranth crops, α-carotene in water spinach was also two to three-fold higher in the crops harvested from the poly-net houses than the open field condition. The iron content of the amaranth varieties produced from the open field conditions was comparable with the previous study (Nordey et al., 2021), but surprisingly it was lower in both the varieties harvested from the poly-net houses. However, the iron supply from the pink poly-net house was comparable with the open field in the winter for both the amaranth crops but pink poly-net house was the highest iron supplier from water spinach in the winter. The poly-net houses were the sole supplier of iron through amaranth crops in the spring, although the niche was shared by open field with the pink poly-net house for the water spinach. It is important to note that only poly-net houses enabled the production of amaranth crops in the spring season, and the water spinach in the winter season.

While comparing pink poly-net house with the white poly-net house, the supply of carotenoids and iron was comparatively better from the pink poly-net house in most of the seasons for all the three crops. A previous study from Japan also demonstrated that red amaranth grown under blue shade polyethylene

TABLE 11 | Climatic conditions [temperature (°C), (Mean ± SD; Min-Max range), and relative humidity (%RH), (Mean ± SD; Min-Max range)] during fall season (Sept 23–Oct 28, 2020), winter season (Dec 9 2020–Feb 8 2021), and spring season (March 24–May 5, 2021).

Treatment	Fall Season		Winter Season		Spring Season	
	T (°C)	%RH	T (°C)	%RH	T (°C)	%RH
Open field	25.7 ± 1.5 (20.8–33.9)	81.5 ± 4.1 (58.8–97.2)	16.1 ± 2.9 (11.0–23.5)	83.8 ± 6.5 (57.6–98.1)	23.2 ± 2.0 (17.9–30.5)	80.7 ± 6.2 (56.2–97.4)
Pink	27.1 ± 4.6 (19.4–37.1)	70.0 ± 13.4 (40.1–90.2)	18.2 ± 5.2 (6.00–33.0)	68.7 ± 14.5 (25.0–90.5)	25.6 ± 5.1 (13.4–37.4)	63.6 ± 14.7 (34.7–87.4)
White	27.2 ± 4.5 (19.5–37.4)	72.3 ± 13.1 (43.4–92.4)	18.4 ± 5.2 (6.6–33.2)	72.0 ± 14.6 (27.5–94.3)	25.8 ± 5.0 (13.9–37.4)	68.6 ± 14.0 (39.9–90.3)

produced more biomass yield with health beneficiary bioactive compounds betacyanins, polyphenol and antioxidant activity during the low temperature regime in spring season (Khandaker et al., 2010). A recent study had assessed the effects of various ratios of combined red, blue, and amber light-emitting diodes on the expression of carotenoid biosynthetic genes and carotenoid accumulation in brassicas (Alrifai et al., 2021). It found that total and individual carotenoids were increased significantly under dose-dependent increasing amber–blue light and decreasing red in most brassica microgreens. According to this study, lipophilic 2,2-diphenyl-1-picrylhydrazyl and ferric reducing antioxidant power antioxidant activities were significantly increased under higher amber and blue light fractions, while oxygen radical absorbance capacity was generally decreased. Hence, the higher blue irradiance inside the pink net house than in the white poly-net house and open field conditions could have involved in the regulatory mechanism of carotenoid biosynthesis in the current study, which requires further validation in future studies.

Based on the RDI values of iron and β-carotene, the number of people who could receive these nutrients from the crops harvested under different production conditions was estimated in the current study. During the off-season (winter for water spinach and spring for amaranths), pink poly-net house was estimated to supply more iron to people than the other production conditions. In all the seasons, the poly-net houses provided more β-carotene than the open field produced crops. Hence, sufficient quantities of iron and β-carotene can be obtained from the leafy vegetables, particularly during the off-season using less cultivation area under protective structures. This is quite important in countries like Taiwan, where the overall share of agricultural land decreased during the last two decades (Chen et al., 2019). However, it should be noted that we made these theoretical estimates based on the RDI values, without considering the bioavailability. Hence, these results should be interpreted in terms of nutrient supply from different crop production conditions, but the actual contribution of these nutrients based on their bioavailability to improving health conditions should be investigated in further studies.

Thus, protected cultivation has demonstrated the supply of nutritious vegetables, especially during the off-season. Since the investment in the construction and maintenance of both the white and pink poly-net houses is similar, use of pink poly-net houses may be considered to produce more nutritious vegetables. Supply of vegetables during the off-season is quite important to bridge the gaps in the seasonal variations in vegetable consumption. In addition, off-season vegetable production can provide better income opportunities to the smallholder farmers. If properly constructed and maintained, protective structures will last longer and reduce the incidence of pests and diseases thus reducing the use of harmful chemical pesticides in vegetable production systems. Thus, protected cultivation is expected to build economic and environmental resilience to the smallholder vegetable producers, while supplying nutritious vegetables to the consumers, especially in the off-season. However, the impacts of different types of protected cultivation on the horticultural and nutritional yield are location- and crop-specific, which can be piloted in new locations before scaling out among smallholder farmers in Asia and Africa for better nutrition and incomes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

SR and PS-C: conceptualization and formal analysis. SR: funding acquisition and writing–original draft. SR, M-YL, W-JW, H-IW, and PS-C: methodology and investigation. M-YL, W-JW, H-IW, and PS-C: writing–review and editing. All authors have read and agreed to the published version of the manuscript.

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