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Effect of shading and high temperature amplitude on yield and phenolic contents of greenhouse capsicum (*Capsicum annuum* L.)

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Abstract

Low temperature is one of the most important abiotic factor that restrict the optimal production of warm-season vegetables in the cold mountain regions. Studies were conducted to establish the possibility of growing capsicum, a temperature sensitive crop, in a naturally ventilated passive solar greenhouse with high temperature amplitude (25.2±2.5ºC). Despite the temperature fluctuation from 6.8±2.9°C at night to 38.6±4.1°C day temperature, flowering and fruiting were seen. The salable yield inside the greenhouse was 4.5 to 4.8-fold higher that of open field. Red shade net combine with the greenhouse technology significantly increased the intercellular CO_2 concentration but affected the photosynthetic rate. Shading delayed flowering and 51-59% reduction in salable fruit yield. Total phenolic contents (TPC) and total flavonoid contents (TFC) of capsicum grown under open field and greenhouse were similar. However, shading reduced the TPC and TFC by 35.2% and 14.6%, respectively inside the greenhouse.

Keywords: Bell pepper; Heat stress; Greenhouse; Protected cultivation; Sweet pepper

1. Introduction

Low temperature is one of the most important abiotic factor that restrict the optimal production of warm-season vegetables in the cold mountain regions, such as the trans-Himalayan Ladakh region. The climatic condition is suitable for growing of cole and root crops, but has sub-optimal heat-units for growing warm-season crops [1]. Use of low-cost passive solar greenhouse is prevalent in the region for growing leafy vegetables during winter. However, overheating is a major problem in such greenhouse during summer, which restrict the use of the greenhouse to spring and winter [2].

Ventilation and shading are helpful ways to lessen heat stress inside the greenhouses. Shade nets reduce the maximum temperature by 1-5ºC depending on shade level and colour of the nets [3-4]. Nets influences the biosynthesis of bioactive compounds [5]. It has also been shown to improve fruit quality and reduce the crop infestation by pests and diseases [6]. Shade nets increases irrigation water use efficiency [7]. The application of shade nets is widespread in tropical and sub-tropical countries for growing vegetables [6], but it has not been studied for improving vegetable production in cold high altitude regions. Shade nets may find application in cold regions when combined with low-cost greenhouse technology [8].

Capsicum is a warm-season crop with a high heat-unit requirement [9]. The optimum temperature for its growth is 20-25°C [10-11], and the optimal root zone temperature is 25-27.5°C [12]. The crop is sensitive to temperature extremes. Low temperature (14°C day/ 15°C night) causes formation of abnormal petals, stamens and gynoecium in the flowers [13]. Low night temperature (14°C or less) reduce number of viable pollen grains and results in deformed fruits [14]. Capsicum, when grown under high temperature has been shown to reduce fruit yield and increase the occurrence of fruit physiological disorders such as blossom-end rot and sunscald, causing significant loss [6, 15-16]. Heat-induced

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flower and fruit abortion can also add to decrease fruit yields [17]. Studies conducted in controlled glasshouse conditions suggested that the day and night temperature difference (i.e. temperature amplitude) is of lesser importance compared with the effect of the 24-h mean temperature for fruit set, development and the growth period of capsicum [18]. However, growth and yield of capsicum in naturally ventilated greenhouses with high day/night temperature amplitude (>20°C) have not been investigated. Capsicum has been reported to respond well to photo-selective shade nets. It enhances plant growth and fruit yield [3, 6, 19]. Red and pearl nets create optimal growing conditions and increase the fruit yield as well as the fruits with fewer physiological disorders [3].

The climate of high altitude mountain regions is not suitable for production of capsicum due to sub-optimal heat-units [1]. Accordingly, the present investigation was directed to determine the possibility of growing capsicum during summer months in a naturally ventilated passive solar greenhouse with high temperature amplitude (>20°C) in the trans-Himalayan Ladakh region. This study was also designed to determine the effects of coloured shade netting combined with greenhouse technology on the photosynthesis, growth, yield, phenolics and flavonoid contents of capsicum.

2. Material and methods

2.1. Study site and growing conditions

Greenhouse and field experiments were conducted in two seasons (2017, 2018). Capsicum (*Capsicum annuum* L.) cv. California Wonder was grown in two naturally ventilated semi-underground greenhouses (Polytrench) and in the open field in trans-Himalayan Ladakh, India (34°08.2'N; 77°34.3'E, elevation 3340 m). Polytrench is a rectangular trench greenhouse (30'×10'×3'; L×W×D) in north-south orientation. It has a tunnel shaped steel frame over the underground trench on which the UV stabilized 120 GSM translucent polyethylene sheet is fixed. A door with an entrance path on the north side allows entry of farm workers inside the greenhouse. Natural ventilation occurs when the door is kept open and the polyethylene sheet is slightly lifted from the south side of the greenhouse [2]. To study the effect of shading, red shade net was placed below the polyethylene sheet in one of the Polytrench greenhouse. Temperature and relative humidity were recorded daily with a hygro-thermometer. The day/night temperature amplitude was calculated as the difference between day and night temperatures. Soil temperature was measured at 10 cm depth using a soil thermometer. Photosynthetically active radiation (PAR) and UV were recorded with a radiometer. The weather data of the greenhouses and open field are shown in Table 1.

2.2. Experimental design

The experiment was conducted under two microclimate conditions- Polytrench greenhouse, shaded greenhouse (Polytrench+red shade net), and open field. Three replications were taken in each condition. Each replication plot was 2.5 × 1.3 m in size, and consisted of 15 plants spaced at 35×40 cm. FYM (3.0 kg per m^2) was applied at the time of field preparation. Seedlings were manually transplanted on 24 May 2017 and 22 May 2018. Flood irrigation was done at three days interval during initial plant establishment followed by one week interval at later stages. Weeding was done twice during the growing season.

2.3. Growth and yield attributes

Plant growth parameters (Table 3) were recorded at 30, 60 and 90 days after transplanting (DAT). Relative growth rate (RGR), leaf weight ratio (LWR), specific leaf area (SLA), leaf weight ratio (LWR), leaf area ratio (LAR) and net assimilation rate (NAR) were determined at 0-30, 30-60, 60-90 DAT. RGR, SLA, LWR and NAR were determined as described by Hunt *et al*. (2002) [20]. Fruits were harvested thrice from mid-August to late September. All fruits that attain greater than 45 gram in weight were considered as salable. Photosynthesis was recorded on fully expanded leaves on a clear sky day on 20 June, 17 July and 29 Aug 2018 using a Portable Photosynthesis System (CIRAS-3, PP Systems, and USA). The data recorded on different dates were pooled.

Table 1 Temperature data of open field, greenhouse and shaded greenhouse during cropping seasons in trans-Himalayan Ladakh region (2017)

Month	Open field (°C)			Greenhouse (°C)			Shaded Greenhouse (°C)			Soil temperature at 12 noon (°C)		
	Max	Min	Amplitude	Max	Min	Amplitude	Max	Min	Amplitude	Open	Greenhouse	Shaded Greenhouse
May	16.4±2.7	4.8±3.2	11.6±2.0	35.3±2.5	6.8±2.9	28.5±2.7	33.4±2.4	8.4±2.5	25.0±2.0	14.0±2.8	18.5±0.7	16.5±0.6
Jun	19.1±4.0	7.6±2.4	11.5±2.7	36.8±3.8	10.1±2.7	26.8±3.6	34.1±4.5	10.9±2.1	23.1±4.6	22.0±1.7	23.3±1.5	18.7±2.3
Jul	25.1±4.0	13.0±2.9	12.0±4.4	38.6±4.1	14.8±1.8	23.7±3.6	32.7±4.2	15.6±1.6	17.1±3.6	23.3±1.7	26.8±3.8	22.3±1.7
Aug	25.8±2.3	12.6±2.2	13.2±1.7	37.6±4.3	13.0±2.5	24.6±3.8	30.5±3.6	14.2±1.9	16.3±2.8	23.5±3.5	24.5±2.1	22.0±1.4
Sept	20.8±2.0	6.8±2.3	14.0±3.1	31.3±2.9	9.2±1.9	22.2±3.7	25.0±1.6	10.4±1.8	14.6±1.8	16.0±1.4	22.0±2.8	17.0±2.8
Mean	21.4±4.0	9.0±3.7	12.5±1.1	35.9±2.8	10.8±3.2	25.2±2.5	31.1±3.7	11.9±2.9	19.2±4.6	19.8±4.4	23.0±3.1	19.3±2.7

Table 2 PAR and UV data of open field, greenhouse and shaded greenhouse during cropping seasons in trans-Himalayan Ladakh region (2017)

Month	PAR (μmol/m²s)			UV-A (mW/cm²)			UV-B (μW/cm²)		
	Open field	Greenhouse	Shaded Greenhouse	Open field	Greenhouse	Shaded Greenhouse	Open field	Greenhouse	Shaded Greenhouse
May	1739.3±288.5	1072.3±177.8	282.1±114.2	4.99±0.12	0.08±0.05	0.03±0.01	14.96±7.01	0.09±0.02	0.07±0.00
Jun	1362.1±571.6	939.7±394.3	268.9±97.8	3.96±2.05	0.10±0.06	0.03±0.01	15.14±8.88	0.17±0.11	0.03±0.02
Jul	1612.7±485.1	1011.4±304.2	269.2±112.4	3.82±1.04	0.09±0.04	0.03±0.01	20.66±5.66	0.21±0.11	0.04±0.02
Aug	1900.7±34.5	1024.7±18.5	282.9±119.0	5.17±0.00	0.17±0.04	0.08±0.04	15.79±4.48	0.29±0.07	0.08±0.02
Sept	1903.4±223.8	1041.4±122.4	259.3±30.3	5.05±0.02	0.12±0.00	0.03±0.01	20.88±1.88	0.36±0.22	0.02±0.00
Mean	1703.6±226.3	1017.9±49.3	272.5±10.0	4.60±0.65	0.11±0.04	0.04±0.02	17.49±3.01	0.22±0.10	0.05±0.03

2.4. Total phenolics and flavonoids assays

Salable fruits at final harvest were homogenized with the help of mortar and pestle, and subjected to two cycles of extraction with methanol. Each sample (200 mg) was extracted for 15 min with 1.5 ml of methanol in a 2 ml micro centrifuge tube and vortexed at room temperature at 1100 rpm. The sample was then centrifuged at 5600 g for 10 min and the supernatant was recovered. The same procedure was repeated for the second cycle of extraction. The supernatant from both the extracts were combined to determine total phenolic contents (TPC) and total flavonoid content (TFC). TPC was determined as described by Singleton and Rossi [21], and expressed as gallic acid equivalent (mg GAE/100g FW). Estimation of the TFC was carried out using the method of Zhishen et al. [22], and expressed as quercetin equivalent (mg QE/100g FW). Total soluble solids (TSS) were measured with refractometer (ATAGO, Tokyo) and values were corrected at 20°C. Titrable acidity (TA) was determined by titration method [23] and expressed as % malic acid.

2.5. Statistical analysis

All the experiments were performed in triplicates. Significance of differences between means was determined by Tukey's test $p \leq 0.05$ level. The experimental results were expressed as mean ± standard deviation (SD) using statistical analysis with SPSS. One way analysis of variance (ANOVA) and post hoc analysis with 2-sided Tukey's HSD at $p \leq 0.05$ level were performed.

3. Results and discussion

3.1. Microclimate inside the greenhouses

The weather data of the open field and the microclimatic conditions inside the two greenhouses are shown in Tables 1 and 2. The mean minimum and maximum temperature of open field were 9.0±3.7 and 21.4±4.0°C, respectively. The mean temperature amplitude was 12.5±1.1°C. The minimum temperature recorded under the open field was significantly low in contrast to the optimum temperature range (20-25°C) for growth of capsicum [10-11]. The mean monthly maximum temperature inside the Polytrench greenhouse varied from 31.3±2.9 to 38.6±4.1°C, while the minimum temperature varied from 9.2±1.9 to 14.8±1.8°C. The mean temperature amplitude was 25.2±2.5°C. The use of shade net lowered the maximum temperature inside the greenhouse by 4.8°C during day time. However, it increases the night temperature by 1.1°C. Therefore, the temperature amplitude inside shaded greenhouse was significantly low (19.2±4.6°C) as compared to the greenhouse without the shade net (25.2±2.5°C). Both the maximum and minimum temperature inside the two greenhouses was much beyond the optimal growth temperature range. The temperature was 23.0±3.1 and 19.3±2.7°C, respectively under Polytrench and shaded greenhouse conditions. A significant difference in PAR was observed between open field and the two greenhouse conditions. The soil temperature under open field was 19.8±4.4°C at noon which was significantly lower than the optimal root zone temperature of 25-27.5°C [12]. The mean PAR at noon in open field was 1703.6±226.3 μmol/m^{2}s as against 1017.9±49.3 and 272.5±10.0 μmol/m^{2}s inside the Polytrench and shaded greenhouses, respectively.

3.2. Growth attributes

Both shading and temperature exhibited a significant effect on plant growth and development (Table 3). Plants height, leaf area and number of leaves were significantly low at suboptimal temperature conditions in open field. Greenhouse conditions increase plant height and number of leaves by 91 and 74%, respectively at 90 DAT. Under shaded greenhouse conditions, plants undergo morphological changes and have greater leaf area, thinner leaves and taller stem compared with plants adapted to strong light. The results are consistent with the earlier reports [8, 24]. Shading reduced the development of lateral shoots on the main stem of the plants, which is consistent with earlier report [19]. Under shaded condition, the number of leaves per plant was significantly low (42.6±6.6) as compared to greenhouse without the shade net (65.2±9.8 at 90 DAT). The results are in agreement with previous report that number of leaves reduced with increasing shading level [24].

The plant RGR describes the rate of increase in plant mass per unit plant mass already present. The RGR of plants grown under open field was significantly low, especially at the early growth stage, as compared to plant grown under greenhouse conditions. At 0-30 DAT the RGR of plant under open field was 13.7±6.3 as against 52.1±12.8 and 52.5±14.1 mg g^{-1} d^{-1} in Polytrench and shaded greenhouses. Under high temperature shaded condition, the RGR showed no significant effect at initial growing stages (0-30 DAT). However, at 60-90 DAT a decrease of 30% in RGR was recorded under shaded condition. Plants had thinner leaves under shaded condition as previously reported [24]. However, no trend was observed in plants grown under low (open field) and high temperature (Polytrench greenhouse) conditions with high PAR. SLA was recorded lowest in plants grown under open field at 0-30 and 60-90 DAT. The NAR was found highest in the greenhouse without shade. Shading reduced the NAR at high temperature conditions by 14-56% depending on growth stages. Under bright sunlight and low temperature condition in open field, the NAR was lowest at 0-30 and 60-90 DAT.

Table 3 Growth parameters of capsicum grown in open field, greenhouse and shaded greenhouse in trans-Himalayan Ladakh region

Growth parameters	DAT	Open field	Greenhouse	Shaded Greenhouse
Plant height (cm)	0	4.9±0.4Aa	4.9±0.4Aa	4.9±0.4Aa
	30	6.7±1.0Aa	11.4±2.3Bb	14.5±2.5Bc
	60	12.6±0.8Ba	30.2±4.5Cb	38.0±7.4Cc
	90	24.5±2.3Ca	46.8±4.5Db	80.3±7.0Dc
Stem diameter (mm)	0	2.0±0.3Aa	2.0±0.3Aa	2.0±0.3Aa
	30	2.6±0.3Aa	3.7±0.7Ab	3.0±0.4Ba
	60	4.8±0.6Ba	8.0±1.4Bb	4.6±0.7Ca
	90	8.5±1.5Ca	10.1±2.0Ca	8.5±0.9Da
Number of leaves	0	4.4±0.9Aa	4.4±0.9Aa	4.4±0.9Aa
	30	5.6±0.5Aa	9.1±2.4Ab	7.2±0.8Aab
	60	15.2±4.7Ba	33.6±7.9Bb	15.3±1.8Ba
	90	37.4±9.2Ca	65.2±9.8Cb	42.6±6.6Ca
Leaf thickness (mm)	0	0.29±0.03Aa	0.29±0.03Aa	0.29±0.03Aa
	30	0.30±0.03ABa	0.36±0.03Bb	0.27±0.04ABa
	60	0.35±0.03BCc	0.29±0.03Ab	0.14±0.02Aa
	90	0.35±0.02Cb	0.34±0.01Bb	0.21±0.09Aa
Leaf area (cm2)	30	16.3±5.8Aa	75.2±21.0Ab	85.3±4.4Ab
	60	346.8±18.9Ba	907.7±250.4Bb	573.2±201.2Bab
	90	796.3±6.4Ca	3104.3±104.2Cb	1653.5±160.3Cab
Root:shoot	30	0.17±0.07Xa	0.16±0.01X a	0.14±0.01Ya
	60	0.20±0.02Xb	0.19±0.01Yb	0.10±0.02Xa
	90	0.18±0.02Xb	0.19±0.01 Yb	0.10±0.01Xa
Lateral shoots (nos)/ plant	60	0.0±0.0a	2.1±0.9b	0.0±0.0a
	90	2.8±0.4a	3.1±0.3a	2.8±0.7a
Buds(nos)/Plant	60	4.6±1.8a	19.1±6.5b	2.1±1.5a
	90	17.4±9.3b	3.6±3.0a	11.7±4.7b
Flower(nos)/plant	60	0.0±0.0a	1.2±1.2b	0.0±0.0a
	90	2.6±1.1ab	1.4±1.3a	4.4±2.1b
Fruit(nos)/ plant	60	0.0±0.0a	1.0±1.0b	0.0±0.0a
	90	1.4±1.1a	4.8±1.5b	2.2±1.5a

RGR (mg g-1 d-1)	0-30	13.7±6.3a	52.1±12.8b	52.5±14.1b
	30-60	96.7±3.4ab	109.4±6.0b	76.6±13.0a
	60-90	41.9±1.5a	48.3±9.0a	52.4±9.6 a
SLA (cm2g-1)	0-30	185.6±66.7a	317.6±107.2 ab	383.0±5.7b
	30-60	223.8±0.3b	166.2±3.6a	280.2±1.1c
	60-90	189.0±9.7a	249.1±77.0a	219.9±9.3a
LWR (g / g-1)	0-30	0.58±0.04a	0.62±0.03a	0.57±0.00 a
	30-60	0.61±0.01c	0.58±0.01b	0.54±0.02a
	60-90	0.50±0.03b	0.33±0.01a	0.46±0.09ab
LAR (cm-2 g-1)	0-30	105.2±30.8a	198.4±74.7a	217.7±2.4a
	30-60	137.5±1.7b	95.9±0.5a	152.0±4.7c
	60-90	94.1±10.0a	82.5±27.4a	100.7±15.7a
NAR (mg cm-2 d-1)	0-30	0.13±0.06a	0.28±0.11a	0.24±0.07a
	30-60	0.70±0.03b	1.14±0.06c	0.50±0.07a
	60-90	0.45±0.06a	0.65±0.29a	0.52±0.0a

Values represented as mean ± SD For each row, different lowercase letters indicate significantly different at p ≤0.05, as measured by Tukey's test between the growing microclimate conditions for each column, different uppercase letters indicate significantly different at p ≤0.05, as measured by Tukey's test between growing periods

3.3. Photosynthetic parameters

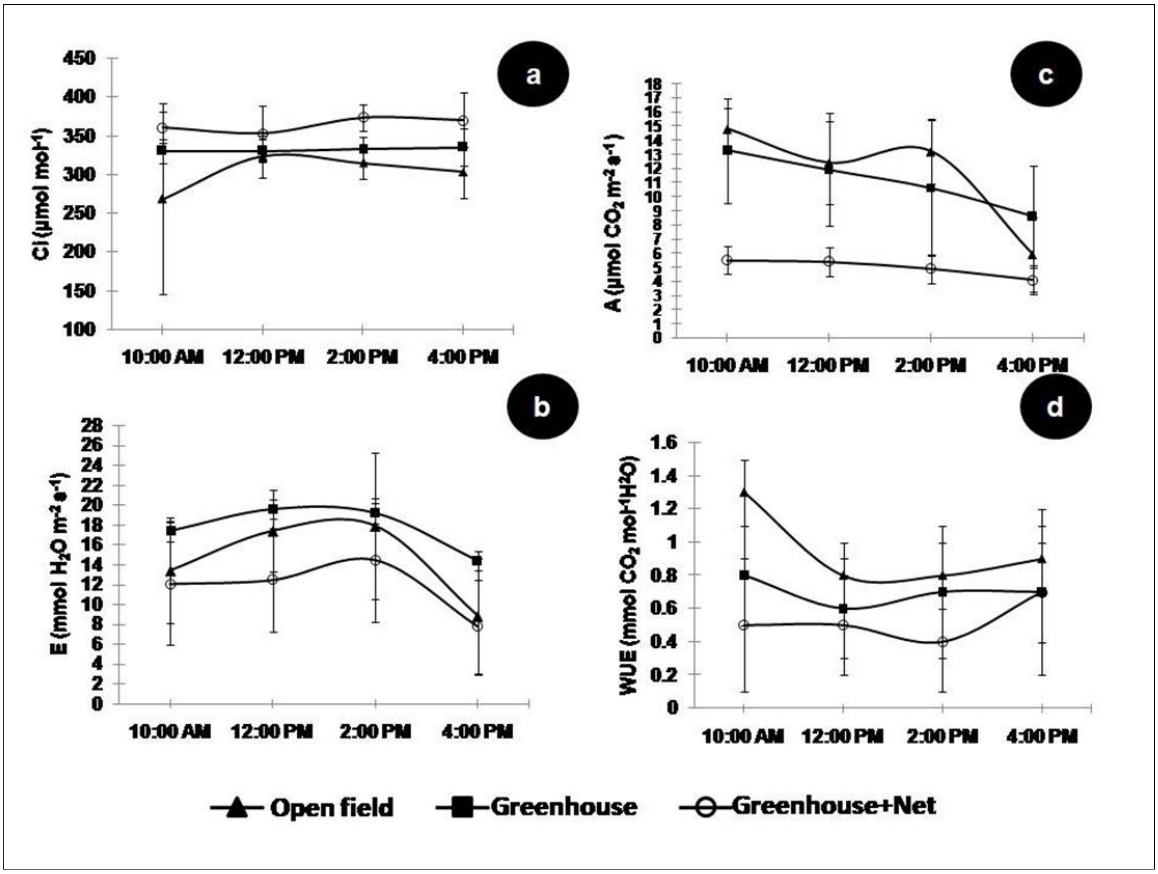

Figure 1 Difference in (a) intercellular CO_2 concentration (Ci); (b) transpiration rate (E); (c) photosynthesis rate (A); and (d) water use efficiency (WUE) of capsicum grown under open field, greenhouse and greenhouse+net at different time intervals

The change in photosynthetic rate (A), intercellular CO_2 concentration (Ci), transpiration rate (E) and water-use efficiency (WUE) measured at different time intervals are shown in Figure 1. Ci did not vary significantly at different time during the day under greenhouse conditions. However, Ci varied significantly at different time intervals in open field. Ci under open field was significantly low as compared to greenhouse conditions. Covering of greenhouse with red shade net increased the crop Ci in the greenhouse.

Photosynthetic rate was recorded the highest under open field except at 4 PM where the highest value was observed in Polytrench greenhouse. Shading significantly affected the photosynthetic rate under greenhouse condition. The result is consistent with with Díaz-Pérez [24], who reported that net photosynthesis decreased with increasing shade level, particularly above 47% shade level. Transpiration rate (E) was recorded highest under Polytrench greenhouse, which may be because of high temperature. Shading significantly reduced the transpiration rate inside the greenhouse, which could be because of poor ventilation due to the shade net. Low temperature under open field resulted in high WUE under open field condition. The result suggested that plants were severely affected by high temperatures. Shading caused a decline in WUE inside greenhouse. Díaz-Pérez [24] also recorded that WUE decreased with increased shade level, particularly above 47% shade level.

3.4. Flowering and fruit yield

Temperature and shading are known to influence flowering, fruit set and fruit growth of capsicum [18-19, 24]. The optimum temperature for capsicum growth is 20-25°C [10-11]. High temperature increase the incidences of fruit physiological disorders causing significant loss [6, 15-16]. Low temperature (14°C day/ 15°C night) causes the formation of abnormal petals and stamens [13]. Night temperature below 14°C reduces the number of viable pollen grains and results in deformed fruits [14]. Heat-induced flower and fruit abortion occurs at high temperature [17]. In the present study microclimatic conditions varying in temperature and shading showed a significant effect on flowering and fruiting in capsicum. Despite the night temperature below 15°C and day temperature above 35°C, flowering and fruiting were observed under the greenhouse conditions (Table 4). Differences in our results with that of previous reports on the effect of temperature on capsicum flowering and fruiting may be due to dissimilarity in growing conditions. Temperature effects have been studied under growth chamber or controlled greenhouse conditions [13-14, 18], while in the present study the same was observed under naturally ventilated greenhouse condition with fluctuating temperature.

Table 4 Capsicum yield in open field, greenhouse and shaded greenhouse in trans-Himalayan Ladakh region

Number of fruit and yield per plant	2017			2018		
	Open	**Greenhouse**	**Shaded Greenhouse**	**Open**	**Greenhouse**	**Shaded Greenhouse**
No. of marketable fruit	$1.9±0.9_{A}^{a}$	$5.9±1.3_{B}^{b}$	$2.8±0.5_{A}^{a}$	$1.9±0.4_{A}^{x}$	$6.8±0.5_{B}^{z}$	$3.7±0.41_{A}^{y}$
Marketable fruit weight (g)	$55.0±9.0_{A}^{a}$	$76.6±9.8_{B}^{b}$	$68.0±3.9_{AB}^{ab}$	$56.2±2.1_{A}^{x}$	$75.0±1.9_{B}^{y}$	$68.8±1.0_{AB}^{z}$
Marketable yield (g)	$98.6±12.4_{A}^{a}$	$444.7±23.5_{D}^{c}$	$180.4±15.2_{B}^{b}$	$107.4±12.1_{A}^{x}$	$516.5±28.5_{E}^{z}$	$251.4±3.5_{C}^{y}$
No. of non-marketable fruit	$0.8±0.2_{A}^{a}$	$0.7±0.2_{A}^{a}$	$0.9±0.5_{AB}^{a}$	$0.9±0.1_{AB}^{y}$	$0.7±0.0_{A}^{x}$	$1.5±0.1_{B}^{z}$
Non-marketable fruit weight (g)	$10.8±1.1_{A}^{a}$	$11.1±1.9_{A}^{a}$	$12.5±8.0_{A}^{a}$	$17.4±0.5_{A}^{z}$	$14.0±0.1_{A}^{y}$	$8.5±0.5_{A}^{x}$
Non-marketable yield (g)	$8.9±2.2_{A}^{a}$	$7.6±3.3_{A}^{a}$	$11.3±5.9_{A}^{a}$	$15.5±2.0_{A}^{x}$	$9.9±0.3_{A}^{x}$	$12.8±0.3_{A}^{y}$

Values represented as mean ± SD For each row, different lowercase letters indicate significantly different at p ≤0.05, as measured by Tukey's test between the growing microclimate conditions

Earlier flower bud formation was observed at 30 DAT under Polytrench greenhouse (19.1±6.5) as against 4.6±1.8 number under open field (Table 3). Delay in flowering under open field may be attributed to low temperature [18] and slow vegetative growth. Shading resulted in 89% reduction in flower bud formation in the greenhouse at 30 DAT. The fruit yield depends on the total number of fruits and fruit size. The number of marketable fruits reduced significantly under open field with low temperature as well as under greenhouse with shade condition (Table 4). Highest number of marketable fruit was observed under Polytrench followed by shaded greenhouse and open field. Shading resulted in 51-59% reduction in marketable yield depending on year. The marketable yield inside the Polytrench was 4.5 and 4.8-fold higher in 2017 and 2018, respectively as compared to open field. Similar trend was observed in the case of fruit size under the microclimatic conditions. Thus, increased fruit yield under the greenhouse was the result of both increased fruit number and fruit size. Excessive shading inside the shaded greenhouse resulted in reduced number of fruit and fruit size, which was probably associated with reduced photosynthesis and allocation of assimilates to fruits and increased allocation of assimilates to vegetative organs [24]. Another important factor that may be attributed to the reduced fruit yield was the lesser number of side branches under shaded condition (Table 3).

Fruits were harvested between 18 Aug-26 Sept in 2017 and 3 Aug-26 Sept in 2018. Crop under open field reached harvestable stage 17 and 29 days later in 2017 and 2018, respectively as compared to greenhouse condition. Similarly, shading delayed crops reaching harvestable stage by 13 and 22 days in 2017 and 2018, respectively under greenhouse condition. Therefore, both shading and low temperature adversely affect the duration of crops to reach salable stage.

3.5. Total phenolics and flavonoid contents

The TPC of capsicum under open field (75.9±8.7) and Polytrench greenhouse (74.2±15.8) were significantly higher than that of shaded greenhouse (48.1±4.0 mg GAE/100 g FW) (Table 5). Similarly, TFC was significantly higher in open field (27.2±8.5) and Polytrench greenhouse (26.6±5.1) as compared to that of shaded greenhouse-grown plants (22.7±3.3 mg QE/100 g FW). The three climatic conditions differ in light intensity, PAR, UV-transmittance, temperature and humidity (Table 1, 2). Higher TPC and TFC in open and greenhouse-grown crop may be due to higher UV-transmittance as that of shaded greenhouse. UV radiation is a known stress factor. Plant produces flavonoids and related phenolic compounds in response to UV radiation [25-26]. Generally, increasing UV-B radiation induces flavonoids and phenolics synthesis [27-18]. Higher PAR may also be a factor contributing to higher TPC and TFC in capsicum grown under open and greenhouse. In bean leaves a high level of PAR has been reported to give a higher concentration of flavonoids than the low level [29]. Decreased level of TFC in shaded condition was observed, which is in agreement with previous studies on beet leaf [30] and tomatoes [8]. Shading significantly reduced the fruit TSS and acidity.

Table 5 Total phenolic contents (TPC), total flavonoid contents (TFC), total soluble solids (TSS) and acidity of capsicum grown under open field, greenhouse and shaded greenhouse

Parameters	Unit	Open	Greenhouse	Shaded Greenhouse
TPC	mg GAE/100g FW	75.9±8.7^{b}	74.2±15.8^{b}	48.1±4.0^{a}
TFC	mg QE/100g FW	27.2±8.5^{b}	26.6±5.1^{b}	22.7±3.3^{a}
TSS	°Brix	4.7±0.3^{b}	4.6±0.5 ^{b}	4.0±0.2^{a}
TA	%	0.40±0.13^{c}	0.30±0.03^{b}	0.13±0.0^{a}

Values represented as mean ± SD For each row, different lowercase letters indicate significantly different at p ≤0.05, as measured by Tukey's test between the growing microclimate conditions

4. Conclusion

Low temperature is one of the most important abiotic factor that restrict the optimal production of warm-season vegetables in the cold mountain regions. The present study suggested that it is feasible to grow capsicum under naturally ventilated passive solar greenhouse with temperature fluctuation from 6.8±2.9°C at night to 38.6±4.1°C day temperature. The salable yield inside the greenhouse was 4.5 to 4.8-fold higher as compared to open field. Shading caused delayed flowering and 51-59% reduction in salable yield inside the greenhouse. There was no significant difference in TPC and TFC in capsicum grown under open field and greenhouse conditions. However, shading reduced the TPC and TFC by 35.2% and 14.6%, respectively.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declared that there is no conflict of interest.

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