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Influence of pruning and micronutrients sprays on carotenoid, ascorbic acid and antioxidant properties of Indian ber (*Ziziphus mauritiana* Lamk.)

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Abstract
Indian ber (*Ziziphus mauritiana* Lamk.) is considered as poor man's fruit, which is a rich source of vitamins and minerals. An experiment on the influence of pruning height and micronutrient sprays on carotenoid, ascorbic acid and antioxidant properties of Indian ber was conducted in the farmers field at Bongaigaon district of Assam, India for two successive years (2020-21 and 2021-22) with two types of Thailand ber variety, *viz.*, Round Green type and Roundish ovate reddish type. Results depicted that heading back at 75 cm from the ground level and spraying of Borax (0.5%) and $ZnSO_4$ (0.5%) were the best pruning height and micronutrient treatment, respectively in terms of carotenoid content and ascorbic acid content of ber fruits. The red type variety performed better in terms of carotenoid (0.33 µg/g), ascorbic acid (68.49 mg/100 g) and antioxidant properties (DPPH scavenging ability of 9.96%), whereas pruning height and micronutrient sprays failed to have significant impact on the antioxidant properties of the fruits.

Keywords: Antioxidant property, ascorbic acid, ber, carotenoid, DPPPH scavenging activity

Introduction

The poor man's fruit ber (*Ziziphus mauritiana* Lamk.) belonging to the family Rhamnaceae is an important fruit crop. It is rich in vitamins A, B and C as well as considered as appetizer and purifier of blood (Bakshi and Singh). This heat and drought tolerant fruit crop perform well in semi-arid and arid situations (Pareek, 1983) [11].

Annual pruning is essential in ber cultivation as fruits are borne in the leaf axil of current season shoots (Kumar *et al.*, 2016) [9]. The positive effect of micronutrients, especially of boron and zinc have been noticed for various vegetative, yield attributing and qualitative parameters. So, the present study was undertaken during 2020-21 and 2021-22 seasons to assess the effect of pruning height and micronutrients in ber.

Materials and Methods

During 2020-21 and 2021-22, the experiment was undertaken in the farmers field situated at Manikpur, Bongaigaon, Assam, India ($26^0$26′51″ North latitude and $90^0$46′47″ East longitude). The soil of the experimental site was strongly acidic with pH value of 5.1. The site was rich in organic carbon (1.03%).

The selected Indian ber variety was 'Thailand ber'. Two types of Thailand ber were considered for the study, *viz.*, V_1: Round green type, V_2: Roundish ovate reddish type. The type of pruning followed was heading back and two different pruning height i.e. P_1: 50 cm from the ground level and P_2: 75 cm from the ground level were considered for the experiment. Pruning operations were carried out immediately after harvest of the fruits during March. Two micronutrient sources, namely Borax (11% B) and zinc sulphate (33% Zn) supplied the micronutrients at various levels, *viz.*, N_0: No micronutrient, N_1: Borax 0.4%, N_2: Borax 0.5%, N_3: $ZnSO_4$ 0.4%, N_4: $ZnSO_4$ 0.5%, N_5: Borax 0.4% + $ZnSO_4$ 0.4%, N_6: Borax 0.4% + $ZnSO_4$ 0.5%, N_7: Borax 0.5% + $ZnSO_4$ 0.4%, N_8: Borax 0.5% + $ZnSO_4$ 0.5%. Micronutrients were sprayed twice, initially at fruit set and finally at 30 days after the first spray.

Following procedures were followed to estimate carotenoid, ascorbic acid and antioxidant properties-

Carotenoid (µg/g): Total carotenoid was estimated by the method advocated by Rodriguez-Amaya (1999) [13]. The formula used for determining total carotenoid content was as under-

$$\text{Total carotenoid content } (\mu g/g) = \frac{\text{absorbance} \times \text{volume (ml)} \times 10}{\text{absorbance coefficient } (2592) \times \text{sample weight (g)}}$$

Ascorbic acid (mg/100 g): The visual titration method was followed to find out the ascorbic acid content of the samples (Sadasivam and Manickam, 1996) [14] The equation used to find out the ascorbic acid content was-

$$\text{Ascorbic acid (mg/100 g)} = \frac{I \times S \times D \times 100}{A \times W}$$

Where,
I = Indophenol reagent used (ml)
S = Ascorbic acid (mg) reacting with the dye (1 ml)
D = Volume of the extract (ml)
A = Aliquot titrated (ml)
W = Weight of sample (g)

Antioxidant activity: DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) scavenging activity was studies to observe the antioxidant activity (Pramai and Jiamyangyuen, 2016) [12]. The absorbance reading of the sample was noted at 517 nm against blank. The absorbance value of control was also measured at the same wavelength of 517 nm. The formula used for calculation was-

$$\text{DPPH Scavenging ability (\%)} = [1-(A_{sample}/A_{control})] \times 100$$

The design of the experiment was Factorial RBD with three replications. Statistical analyses were performed following standard procedures (Panse and Sukhatme, 1985) [10].

Results and Discussion

Most of the qualitative parameters under study were influenced by the height of pruning and the spraying treatments with micronutrients. In some cases, variations among the varieties were also recorded.

Carotenoid

The variations in pruning height, micronutrient sprays and variety had significant impact on fruit carotenoid content. Table 1 implied that the fruits of reddish variety (V_2) exhibited significantly higher carotenoid content (0.33 µg/g) compared to greenish variety V_1 (0.24 µg/g). Carotenoid content decreased with the severity of pruning. Pruning intensity P_1 recorded significantly lower carotenoid content (0.27 µg/g) than P_2 (0.30 µg/g). More penetration of light to the fruit might be the cause for this result as light is very important for carotenoid synthesis in fruits (Tao *et al.*, 2003) [16]. Among various levels of micronutrient sprays, N_7 and N_8 produced fruits with significantly higher carotenoid content (0.30 µg/g) while the lowest value of 0.27 µg/g was seen in N_0 and N_1. Increase in carotenoid content as a result of boron application was also suggested (Hanafy *et al.*, 2009) [7]. Increased carotenoid content in fruits as a result of zinc application was reported by Zaman *et al.* (2019) [17]. Significant diversity in fruit carotenoid levels were also observed in various interactions (Table 2). The carotenoid content (0.36 µg/g) in fruits produced at interaction levels $V_2P_2N_7$ and $V_2P_2N_8$ was significantly higher compared to other levels of interaction. The least amount of carotenoid was noted in $V_1P_1N_0$ (0.20 µg/g). Pool effect of pruning height, micronutrient and variety also forced significant change in the level of fruit carotenoid in interactions.

Ascorbic acid

All the three factors, *viz.*, variety, height of pruning and levels of micronutrient sprays exhibited significant effect on ascorbic acid content of fruits (Table 1). Among the two types of varieties, fruits of V_2 contained significantly higher ascorbic acid (68.49 mg/100 g) compared to V_1 (66.82 mg/100 g). Similar kind of findings were also reported by Ghosh and Mathew (Ghosh and Mathew, 2002) [6]. Heading back at a height of 75 cm (P_2) depicted significantly higher ascorbic acid content of 68.62 mg/100 g compared to heading back at a height of 50 cm (P_1), where ascorbic acid content of 66.69 mg/100 g was recorded. It might be attributed to pruning operations, which made more nutrients available for the developing fruits (Singh *et al.*, 2001) [15]. Among various combinations of micronutrient sprays, N_8 recorded the highest ascorbic acid (68.55 mg/100 g) and the lowest amount of 66.77 mg/100 g in control N_0. The content of ascorbic acid was influenced positively by boron application (Hosein-Beigi *et al.*, 2019) [8]. The catalytic enzymes and co-enzymes involved in ascorbic acid production, were synthesized rapidly and in more quantity by zinc and hence zinc application exhibits positive impact in ascorbic acid content of fruits (Chandra and Singh, 2015) [3]. Further, boron is found to increase the zinc uptake and transport (Ahmed *et al.*, 2011) [1], which leads to increased quantity of fruit ascorbic acid content. As depicted in Table 2, interaction effects also differed significantly in ascorbic acid content because of significant effect of all the three factors.

Antioxidant property

The DPPH scavenging ability (%) was measured to study antioxidant property of fruits. Table 2 revealed that variety had noteworthy effect on the percentage of DPPH scavenging ability. The reddish variety V_2 exhibited significantly superior DPPH scavenging ability (9.96%) compared to greenish V_1 (9.19%). Pruning levels and micronutrient treatments did not have significant effect on DPPH scavenging ability. The interaction effects presented in Table 3 reveals significant difference among different interactions. The highest value of DPPH scavenging ability was noted in $V_2P_1N_4$ (10.05%), whereas the lowest level was found in $V_1P_1N_8$ (9.15%). The amount of total carotenoid, vitamin C and phenolic compound are considered as the determining factor of antioxidant activity (Gardner *et al.*, 2000) [5]. Presence of higher quantity of carotenoid and ascorbic acid in reddish type variety might led to higher DPPH scavenging ability (Edge *et al.*, 1997) [4]. The varietal influence led to significant deviation in interaction effects.

Table 1: Carotenoid and ascorbic acid content as influenced by variety, pruning height and micronutrient sprays

Treatments	Carotenoid (□g/g)			Ascorbic acid (mg/100 g)		
	2020-21	2021-22	Pooled	2020-21	2021-22	Pooled
V_1	0.24	0.24	0.24	66.99	66.65	66.82
V_2	0.33	0.33	0.33	68.55	68.43	68.49
CD (0.05)	0.003	0.002	0.002	0.109	0.106	0.081
P_1	0.27	0.27	0.27	66.81	66.57	66.69
P_2	0.30	0.30	0.30	68.72	68.51	68.62
CD (0.05)	0.003	0.002	0.002	0.109	0.106	0.081
N_0	0.27	0.27	0.27	66.93	66.61	66.77
N_1	0.27	0.27	0.27	67.11	66.90	67.00
N_2	0.28	0.27	0.28	67.29	67.09	67.19
N_3	0.28	0.28	0.28	67.46	67.30	67.38
N_4	0.29	0.29	0.29	67.80	67.62	67.71
N_5	0.29	0.29	0.29	67.99	67.78	67.89
N_6	0.29	0.29	0.29	68.29	67.94	68.12
N_7	0.30	0.30	0.30	68.41	68.18	68.29
N_8	0.30	0.30	0.30	68.64	68.45	68.55
CD (0.05)	0.007	0.005	0.005	0.231	0.225	0.171

Table 2: Carotenoid and ascorbic acid content as influenced by interaction of variety, pruning height and micronutrient sprays

Treatments	Carotenoid (µg/g)			Ascorbic acid (mg/100 g)		
	2020-21	2021-22	Pooled	2020-21	2021-22	Pooled
T_1: $V_1P_1N_0$	0.20	0.20	0.20	64.86	64.58	64.72
T_2: $V_1P_1N_1$	0.20	0.21	0.21	65.07	64.93	65.00
T_3: $V_1P_1N_2$	0.21	0.21	0.21	65.35	65.18	65.27
T_4: $V_1P_1N_3$	0.22	0.21	0.22	65.58	65.34	65.46
T_5: $V_1P_1N_4$	0.22	0.22	0.22	66.01	65.70	65.85
T_6: $V_1P_1N_5$	0.23	0.22	0.23	66.11	65.97	66.04
T_7: $V_1P_1N_6$	0.23	0.23	0.23	66.69	66.00	66.35
T_8: $V_1P_1N_7$	0.24	0.23	0.24	66.75	66.43	66.59
T_9: $V_1P_1N_8$	0.25	0.23	0.24	67.00	66.65	66.83
T_{10}: $V_1P_2N_0$	0.25	0.24	0.24	67.19	66.80	67.00
T_{11}: $V_1P_2N_1$	0.25	0.24	0.25	67.47	67.00	67.24
T_{12}: $V_1P_2N_2$	0.25	0.24	0.25	67.62	67.12	67.37
T_{13}: $V_1P_2N_3$	0.26	0.25	0.26	67.71	67.30	67.51
T_{14}: $V_1P_2N_4$	0.26	0.26	0.26	68.03	67.77	67.90
T_{15}: $V_1P_2N_5$	0.27	0.27	0.27	68.32	67.98	68.15
T_{16}: $V_1P_2N_6$	0.27	0.27	0.27	68.50	68.12	68.31
T_{17}: $V_1P_2N_7$	0.27	0.27	0.27	68.67	68.32	68.49
T_{18}: $V_1P_2N_8$	0.28	0.28	0.28	68.84	68.58	68.71
T_{19}: $V_2P_1N_0$	0.30	0.30	0.30	67.07	66.72	66.89
T_{20}: $V_2P_1N_1$	0.30	0.30	0.30	67.17	66.95	67.06
T_{21}: $V_2P_1N_2$	0.31	0.31	0.31	67.30	67.16	67.23
T_{22}: $V_2P_1N_3$	0.31	0.31	0.31	67.45	67.33	67.39
T_{23}: $V_2P_1N_4$	0.31	0.31	0.31	67.68	67.62	67.65
T_{24}: $V_2P_1N_5$	0.31	0.32	0.32	67.86	67.66	67.76
T_{25}: $V_2P_1N_6$	0.32	0.32	0.32	68.01	67.86	67.93
T_{26}: $V_2P_1N_7$	0.32	0.32	0.32	68.15	68.03	68.09
T_{27}: $V_2P_1N_8$	0.33	0.33	0.33	68.52	68.21	68.36
T_{28}: $V_2P_2N_0$	0.33	0.33	0.33	68.58	68.35	68.46
T_{29}: $V_2P_2N_1$	0.34	0.33	0.34	68.73	68.70	68.72
T_{30}: $V_2P_2N_2$	0.34	0.34	0.34	68.88	68.89	68.89
T_{31}: $V_2P_2N_3$	0.34	0.34	0.34	69.10	69.25	69.17
T_{32}: $V_2P_2N_4$	0.35	0.35	0.35	69.48	69.37	69.43
T_{33}: $V_2P_2N_5$	0.35	0.35	0.35	69.66	69.53	69.60
T_{34}: $V_2P_2N_6$	0.35	0.35	0.35	69.96	69.80	69.88
T_{35}: $V_2P_2N_7$	0.36	0.36	0.36	70.08	69.92	70.00
T_{36}: $V_2P_2N_8$	0.36	0.36	0.36	70.21	70.35	70.28
CD (0.05)	**0.014**	**0.009**	**0.009**	**0.462**	**0.450**	**0.342**

Table 3: DPPH scavenging ability (%) as influenced by variety, pruning height and micronutrient sprays

Treatments	2020-21	2021-22	Pooled
V_1	9.19	9.19	9.19
V_2	9.95	9.97	9.96
CD (0.05)	0.040	0.041	0.031
P_1	9.57	9.58	9.57
P_2	9.57	9.59	9.58
CD (0.05)	NS	NS	NS
N_0	9.56	9.59	9.57
N_1	9.54	9.55	9.55
N_2	9.58	9.57	9.58
N_3	9.59	9.55	9.57
N_4	9.60	9.60	9.60
N_5	9.59	9.59	9.59
N_6	9.61	9.59	9.60
N_7	9.55	9.59	9.57
N_8	9.51	9.60	9.56
CD (0.05)	NS	NS	NS

Table 4: DPPH scavenging ability (%) as influenced by interaction of variety, pruning height and micronutrient sprays

Treatments	2020-21	2021-22	Pooled
T_1: $V_1P_1N_0$	9.19	9.19	9.19
T_2: $V_1P_1N_1$	9.14	9.22	9.18
T_3: $V_1P_1N_2$	9.22	9.25	9.23
T_4: $V_1P_1N_3$	9.20	9.11	9.16
T_5: $V_1P_1N_4$	9.20	9.14	9.17
T_6: $V_1P_1N_5$	9.24	9.24	9.24
T_7: $V_1P_1N_6$	9.24	9.16	9.20
T_8: $V_1P_1N_7$	9.19	9.13	9.16
T_9: $V_1P_1N_8$	9.15	9.14	9.15
T_{10}: $V_1P_2N_0$	9.16	9.19	9.18
T_{11}: $V_1P_2N_1$	9.20	9.23	9.21
T_{12}: $V_1P_2N_2$	9.24	9.17	9.20
T_{13}: $V_1P_2N_3$	9.17	9.22	9.20
T_{14}: $V_1P_2N_4$	9.16	9.24	9.20
T_{15}: $V_1P_2N_5$	9.21	9.18	9.20
T_{16}: $V_1P_2N_6$	9.20	9.21	9.21
T_{17}: $V_1P_2N_7$	9.19	9.24	9.21
T_{18}: $V_1P_2N_8$	9.18	9.23	9.21
T_{19}: $V_2P_1N_0$	9.98	9.97	9.98
T_{20}: $V_2P_1N_1$	9.85	9.95	9.90
T_{21}: $V_2P_1N_2$	9.80	9.94	9.87
T_{22}: $V_2P_1N_3$	10.03	9.89	9.96
T_{23}: $V_2P_1N_4$	10.08	10.01	10.05
T_{24}: $V_2P_1N_5$	10.07	10.01	10.04
T_{25}: $V_2P_1N_6$	10.01	10.02	10.01
T_{26}: $V_2P_1N_7$	9.88	10.00	9.94
T_{27}: $V_2P_1N_8$	9.79	10.00	9.89
T_{28}: $V_2P_2N_0$	9.92	9.99	9.95
T_{29}: $V_2P_2N_1$	9.98	9.82	9.90
T_{30}: $V_2P_2N_2$	10.07	9.94	10.01
T_{31}: $V_2P_2N_3$	9.95	9.96	9.95
T_{32}: $V_2P_2N_4$	9.97	10.01	9.99
T_{33}: $V_2P_2N_5$	9.82	9.94	9.88
T_{34}: $V_2P_2N_6$	10.00	9.97	9.98
T_{35}: $V_2P_2N_7$	9.95	9.99	9.97
T_{36}: $V_2P_2N_8$	9.93	10.03	9.98
CD (0.05)	**0.161**	**0.190**	**0.122**

Conclusion

From the two years of experimentation, it was clear that all the factors, *viz*., variety, height of pruning as well as micronutrient sprays had significant influence on carotenoid content and ascorbic acid content of ber fruit. The antioxidant property of the fruits was influenced significantly by the variety only, whereas the other two factors did not have any significant influence. Among the two varieties, reddish variety (V_2) was found to be significantly superior to green variety (V_1) in terms of carotenoid content, amount of ascorbic acid and antioxidant activity. Significantly higher amount of carotenoid and ascorbic acid content were detected in the pruning height of 75 cm from the ground level (P_2). Among the treatments with micronutrients, N_8 *i.e*. spraying with Borax 0.5% and $ZnSO_4$ 0.5% proved to be the best in terms of carotenoid and ascorbic acid content.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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