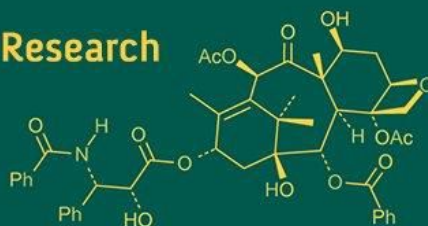


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Impact assessment of physical and cultural interventions on frost injury and stress-responsive biochemical traits in mulberry saplings under temperate conditions

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Abstract

Frost is a major abiotic constraint limiting the survival and early establishment of mulberry (*Morus* spp.) in temperate sericulture regions such as Jammu and Kashmir. Juvenile saplings, particularly those recently transplanted to nursery beds, suffer severe freezing injury that delays growth recovery and reduces foliar biomass. The present investigation evaluated the effectiveness of frost-mitigating cultural and physical interventions by assessing frost injury, growth traits, and stress-responsive biochemical and antioxidant parameters in two mulberry varieties, Goshorami (V₁) and Kokuso-21 (V₂), during 2022-23 and 2023-24. Five treatments were studied: control (T₀), smoke application (T₁), tender shoot protection with insulating polythene (T₂), sapling covering with plastic bottle (T₃), and split fertilizer application (T₄). Significant variation was observed among treatments for all traits. Polythene insulation (T₂) consistently resulted in the lowest frost damage (9.23% in V₁; 6.11% in V₂) and the highest bud sprouting (88.27% and 90.89%), leaf area, shoot length (118.25 cm and 100.94 cm), and biomass attributes, primarily due to reduced radiative heat loss. In contrast, split fertilizer application (T₄) produced the most superior root traits and the maximum accumulation of soluble proteins, sugars, phenols, proline, chlorophyll pigments, and antioxidant activity including FRAP, SOD, PPO, and catalase, indicating nutrient-supported tissue maturity and stronger activation of stress-responsive pathways under natural frost exposure. Overall, physical insulation through T₂ proved most effective in minimizing frost injury and promoting early spring growth, whereas T₄ maximized physiological and biochemical resilience.

Keywords: Kokuso-21, frost, mulberry saplings, goshorami, abiotic stress, sericulture

Introduction

Frost represents one of the most detrimental abiotic stress factors affecting global agriculture, imposing severe limitations on plant growth, geographical distribution, and overall productivity. As temperatures drop below freezing, cellular structures and metabolic functions are disrupted, leading to reduced crop quality and quantity and contributing to yield losses reported as high as 70% in many economically important crops (Shammi *et al.*, 2023; Tschurr *et al.*, 2023; Kumar, 2020) [20, 23, 18]. This makes frost a persistent threat to food security and ecosystem stability in regions prone to extreme winter conditions.

In the Union Territory of Jammu and Kashmir (J&K), frost poses a particularly serious challenge for mulberry (*Morus* spp.), a cornerstone species supporting the sericulture industry. During winter, mulberry plants undergo natural dormancy; however, sub-zero temperatures simultaneously inflict substantial damage on juvenile saplings grown under polyhouse conditions which are subsequently transplanted to nursery beds. Even mature field-grown mulberry plants also suffer frost injury. Juvenile saplings often lose nearly half of their total height due to frost-induced necrosis of their tender upper portions (Baqual *et al.*, 2012) [2]. This setback not only prolongs their growth cycle but also results in reduced leaf biomass, ultimately impacting silkworm rearing efficiency and cocoon productivity thus putting sericulture farmers to tremendous loss. To counter frost injury, several field-based protective techniques have been developed across different crops. Many of these approaches focus on modifying the microclimate to reduce cold stress.

Mechanical methods such as using wind machines or turbines-promote mixing of warm and cold air layers to prevent cold air stagnation around plants (Kalma *et al.*, 1992) ^[16]. Traditional strategies such as creating smoke blankets by burning organic waste materials form an insulating layer that limits long wave radiative heat loss and slightly elevates orchard temperature, thereby reducing frost severity. Similarly, covering plants or branches with insulating materials has proven effective in providing a protective barrier against cold winds and freezing temperatures (Kazemi *et al.*, 2018) ^[17]. While these techniques are widely applied in certain horticultural systems, their systematic evaluation for mulberry remains limited.

Although mulberry is economically important crop raised exclusively for its foliage being used as a feed for silkworms, however research on frost mitigation in this crop remains limited. In view of its added utility in various other areas like mulberry jam, mulberry tea and other cocoon by product use, the study on the frost damage protection assumes importance that too under temperate climatic conditions where temperature generally reaches to sub-zero levels thus providing congenial conditions for frost incidence.

Materials and Methods

Experimental Site and Plant Material

The study was carried out during 2022-23 and 2023-24 at the Division of Host Plant Production, College of Temperate Sericulture, Mirgund, SKUAST-Kashmir. The experiment involved two varieties of mulberry, Goshwerami (V₁) and Kokuso-21 (V₂). Cuttings collected from 8-9 months old shoots were surface-sterilized with 0.1% carbendazim and planted individually in polytubes. The cuttings were maintained in a zero-energy polyhouse for approximately three months, after which uniform saplings were transplanted to the nursery in mid-June following a Randomized Completely Block Design (RCBD). The experiment consisted of five treatments: T₀ (control for each variety), T₁ (microclimate modification through smoke), T₂ (tender shoot covered with insulating bag/black polythene), T₃ (sapling covered with plastic bottle), and T₄ (split fertilizer application), with three replications per treatment and 30 saplings per treatment per replication.

Treatment Application

Recommended fertilizer dose was applied in two splits into 40N:12.5P:12.5K in the second week of June and 10N:12.5P:12.5K in the second week of July for T₄. Smoke and protective coverings were initiated with the onset of freezing temperatures and removed during daytime to prevent excess humidity.

Parameters Recorded

After transplanting the saplings in the nursery, they continue to grow until September, after which they enter winter dormancy and become exposed to naturally occurring low-temperature stress. Observations were recorded in spring following this period of natural cold exposure. Growth and yield parameters included frost damage (%), assessed weekly from December to March, and bud sprouting, recorded during early spring. The number of leaves per sapling was determined from three randomly selected plants, and leaf area was measured using a leaf area meter from

bottom, middle, and top leaves. Leaf moisture content was estimated based on the difference between fresh and oven-dry weights. Additional leaf parameters such as fresh and dry leaf weights were also recorded. Shoot growth was assessed by measuring shoot length, fresh and dry shoot weights, and shoot diameter at three positions (base, middle, and top). Root parameters included the number of roots, fresh and dry root weights, and the length of the longest root. Root volume was determined separately using the water displacement method.

Biochemical parameters were analyzed from fresh leaf samples collected during spring. Soluble protein content was estimated using the Bradford method (1976) ^[7], total soluble sugars by Dubois *et al.* (1956) ^[9], and total phenols following Singleton and Rossi (1965) ^[21]. Proline was quantified according to Bates *et al.* (1973) ^[4], while total chlorophyll was estimated using the DMSO method described by Hiscox and Israelstam (1979) ^[14]. Antioxidant assays included DPPH radical scavenging activity as per Brand-Williams *et al.* (1995) ^[8] and FRAP assay following Benzie and Strain (1996). Enzymatic antioxidant activities were determined for superoxide dismutase (SOD) following Beauchamp and Fridovich (1971) ^[5], Polyphenol Oxidase (PPO) by the method of Augustin *et al.* (1985) ^[1], and catalase activity as described by Barber (1980) ^[3].

Results and Discussion

Frost Damage (%)

Frost injury varied significantly across frost-protection interventions in both mulberry varieties (Table 1). On the final observation date of 01-03-2023/24, the lowest frost damage occurred in T₂ (polythene covering), where damage was only 9.23% in Goshwerami and 6.11% in Kokuso-21. This was followed by T₄ (split fertilizer application), recording 21.06% and 14.86%, respectively. Smoke treatment (T₁) and plastic bottle covering (T₃) exhibited intermediate levels of frost injury, while the highest frost damage was recorded in the control (T₀), where damage reached 31.96% in Goshwerami and 22.63% in Kokuso-21.

The superior performance of polythene can be attributed to its ability to reduce radiative heat loss and create a warmer boundary layer around the tender shoot tips, thereby minimizing frost damage (Kazemi *et al.*, 2018) ^[17]. Smoke also contributed moderate protection, likely through the formation of a temporary thermal blanket that reduces long-wave radiation loss (Kalma *et al.*, 1992) ^[16]. Although T₄ lacked a physical barrier, reduced frost injury compared to the control may be attributed to improved tissue vigor and partially enhanced cold tolerance due to better nutrient status (Waraich *et al.*, 2012) ^[24]. Control saplings experienced maximum necrosis due to full exposure to freezing temperatures.

Bud Sprouting (%)

A significant improvement in bud sprouting was recorded under frost-protection treatments (Table 2). T₂ exhibited the highest bud sprouting, reaching 88.27% in Goshwerami and 90.89% in Kokuso-21. This was followed by T₄, with 77.44% and 82.14%, respectively. T₁ and T₃ resulted in moderate sprouting, while the lowest sprouting was observed in T₀ (65.54% and 74.37%). The higher sprouting in T₂ was due to reduced terminal bud damage under physical insulation, while improved nutrient reserves in T₄

may have facilitated earlier activation of dormant buds (Garcez Neto *et al.*, 2012) ^[10].

Leaf Parameters

Leaf development showed significant treatment effects (Table 3). T₂ recorded the highest number of leaves, with 32.95 leaves in Goshierami and 26.89 in Kokuso-21. Leaf area was also maximum in T₂ (187.91 cm² and 165.66 cm²). Fresh and dry leaf weights were highest in T₂ as well. T₄ followed T₂ in all leaf parameters, while T₁ and T₃ showed moderate leaf growth. The lowest leaf development occurred in T₀, which had only 166.05 cm² and 149.81 cm² leaf area in the two varieties.

The strong performance of T₂ is explained by minimal shoot injury and uninterrupted canopy recovery in spring. T₄ also performed well due to enhanced nutrient assimilation supporting better spring foliage growth (Millard and Grelet, 2010) ^[19]. Leaf moisture content remained statistically non-significant among treatments.

Shoot Parameters

Shoot length, fresh and dry shoot weight, and shoot diameter differed significantly among treatments (Table 4). T₂ recorded the longest shoots, with 118.25 cm in Goshierami and 100.94 cm in Kokuso-21, along with the highest shoot biomass. T₄ followed, while T₁ and T₃ produced moderate shoot growth. T₀ recorded the lowest shoot length, reaching only 100.19 cm in Goshierami and 84.79 cm in Kokuso-21.

Although T₄ initially enhanced shoot growth before winter, its shoots later experienced frost damage due to lack of a physical barrier. In contrast, polythene coverings in T₂ prevented shoot necrosis and ensured maximum shoot elongation, consistent with earlier reports of frost-protection structures in woody crops (Snyder and de Melo-Abreu, 2005) ^[22].

Root Parameters

Unlike shoot and leaf traits, root parameters followed a different trend (Table 5). T₄ recorded the highest number of roots, with 25.10 roots in Goshierami and 20.92 in Kokuso-21. Fresh and dry root weights, and root volume were also highest in T₄. T₂ showed the next highest root development, followed by T₁ and T₃, while T₀ exhibited the lowest root performance.

This is because fertilizer was applied before winter. Although frost damaged the shoots in T₄, roots-protected underground continued to grow under favourable conditions without sustaining any damage, even though the above-ground shoots were affected by frost. Nitrogen enrichment is known to stimulate root proliferation and biomass accumulation in woody perennials (Jiang *et al.*, 2021) ^[15].

Biochemical Parameters

Biochemical traits including soluble proteins, sugars, phenols, and proline recorded significantly higher values in T₄ (Table 6). Soluble protein, for example, reached 26.23 mg/g in Goshierami and 27.90 mg/g in Kokuso-21, with similar trends observed for sugars, phenols, and proline. T₉ and T₇ followed, while the lowest biochemical contents were recorded in T₀.

The higher biochemical accumulation in T₄ can be attributed to the improved physiological maturity achieved due to split fertilizer application (40N in June and 10N in July). While all other treatments received the full recommended fertilizer dose (50N:25P:25K) at once in June, the extended nitrogen supply in T₄ allowed the saplings to mature gradually, resulting in fewer tender tissues by late autumn. Mature tissues are physiologically more capable of synthesizing stress-responsive metabolites following exposure to freezing temperatures. Additionally, because saplings under T₄ experienced greater frost injury than the physically protected T₂ plants, their stress-induced biochemical pathways such as osmotic adjustment, protein synthesis, phenolic accumulation, and proline biosynthesis were more strongly activated. However, this enhancement was insufficient to provide protection comparable to that observed in T₂. These responses align with earlier findings that proteins, soluble sugars, phenolic compounds, and proline act as key osmoprotectants and cryoprotectants in cold-stressed plants (Guy, 1990; Xin and Browse, 2000; Hayat *et al.*, 2012) ^[12, 25, 13]. In contrast, biochemical induction was comparatively lower in T₂ due to reduced frost injury from polythene insulation, resulting in minimal activation of stress-driven metabolic pathways.

Chlorophyll Content

Chlorophyll-a, chlorophyll-b, and total chlorophyll also exhibited maximum values in T₄ (Table 7), with total chlorophyll reaching 1.91 mg/g in Goshierami and 2.15 mg/g in Kokuso-21. These were followed by T₉ and T₇, whereas T₀ recorded the lowest pigment contents.

The enhanced chlorophyll content in T₄ is directly related to the improved nitrogen availability provided by the split fertilizer application. Chlorophyll biosynthesis is strongly dependent on nitrogen status, and the extended availability of nitrogen into July likely supported superior chloroplast development and pigment formation (Millard and Grelet, 2010) ^[19]. Additionally, the T₄ triggered stronger antioxidant activation, which may have aided in chloroplast protection and recovery during spring. In contrast, the physically insulated T₂ saplings suffered minimal frost stress, and therefore exhibited lower induction of chlorophyll-protective biochemical responses, explaining their comparatively lower pigment content.

Antioxidant Potential

Antioxidant activity including DPPH, FRAP, SOD, PPO, and catalase also peaked in T₄ (Table 8). FRAP activity, for instance, reached 22.66 mg TE/g in Goshierami and 24.03 mg TE/g in Kokuso-21, and similar increases were recorded for SOD and catalase enzymes. These were followed by T₉ and T₇, while the lowest antioxidant activities were observed in T₀.

The strong antioxidant response in T₄ reflects the combined effect of higher nutrient availability and greater frost-induced oxidative stress, both of which stimulate the production of enzymatic antioxidants. Elevated SOD and catalase activities are characteristic responses in plants exposed to freezing temperatures, as these enzymes detoxify reactive oxygen species generated during stress (Gill and Tuteja, 2010) ^[11].

Table 1: Extent of frost damage (%) in mulberry saplings under different frost protection interventions

Parameters and Treatments	15-12-2022/23		01-01-2023/24		15-01-2023/24		01-02-2023/24		15-02-2023/24		01-03-2023/24	
	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)
T ₀	18.77 (4.44) ^c	13.28 (3.77) ^c	21.32 (4.72) ^c	15.09 (4.01) ^c	25.08 (5.10) ^c	17.76 (4.33) ^c	28.83 (5.46) ^c	20.41 (4.62) ^c	31.01 (5.65) ^c	21.95 (4.79) ^c	31.96 (5.74) ^c	22.63 (4.86) ^c
T ₁	15.51 (4.06) ^c	10.81 (3.43) ^c	17.65 (4.31) ^c	12.29 (3.64) ^c	20.77 (4.66) ^c	14.46 (3.93) ^c	23.84 (4.98) ^c	16.62 (4.19) ^c	25.65 (5.16) ^c	17.86 (4.34) ^c	26.43 (5.23) ^c	18.43 (4.40) ^c
T ₂	5.40 (2.53) ^a	3.57 (2.13) ^a	6.15 (2.67) ^a	4.09 (2.25) ^a	7.26 (2.87) ^a	4.79 (2.40) ^a	8.32 (3.05) ^a	5.51 (2.55) ^a	8.95 (3.15) ^a	5.92 (2.63) ^a	9.23 (3.19) ^a	6.11 (2.66) ^a
T ₃	16.68 (4.20) ^d	11.99 (3.60) ^d	18.97 (4.46) ^d	13.63 (3.82) ^d	22.34 (4.83) ^d	16.07 (4.13) ^d	25.64 (5.16) ^d	18.45 (4.41) ^d	27.59 (5.34) ^d	19.86 (4.56) ^d	28.45 (5.42) ^d	20.44 (4.63) ^d
T ₄	12.37 (3.65) ^b	8.72 (3.11) ^b	14.04 (3.87) ^b	9.91 (3.30) ^b	16.53 (4.18) ^b	11.69 (3.56) ^b	18.97 (4.46) ^b	13.42 (3.79) ^b	20.44 (4.63) ^b	14.45 (3.93) ^b	21.06 (4.69) ^b	14.86 (3.98) ^b
C.D ($p \leq 0.05$)	0.046	0.039	0.029	0.009	0.106	0.122	0.063	0.033	0.083	0.045	0.011	0.013

* Each value is the Mean of three separate observations.

T₀ (control for each variety), T₁ (microclimate modification through smoke), T₂ (tender shoot covered with insulating bag/black polythene), T₃ (sapling covered with plastic bottle), and T₄ (split fertilizer application).

Table 2: Effect of frost protection techniques on bud sprouting (%) of mulberry saplings under nursery conditions

Parameters and Treatments	Bud sprouting (%)	
	V1 (Goshoerami)	V2 (Kokuso-21)
T ₀	65.54 (8.15) ^c	74.37 (8.68) ^c
T ₁	71.07 (8.48) ^c	78.57 (8.92) ^c
T ₂	88.27 (9.44) ^a	90.89 (9.58) ^a
T ₃	69.05 (8.37) ^d	76.56 (8.80) ^d
T ₄	77.44 (8.85) ^b	82.14 (9.11) ^b
C.D ($p \leq 0.05$)	0.024	0.028

* Each value is the Mean of three separate observations.

T₀ (control for each variety), T₁ (microclimate modification through smoke), T₂ (tender shoot covered with insulating bag/black polythene), T₃ (sapling covered with plastic bottle), and T₄ (split fertilizer application)

Table 3: Effect of frost protection techniques on leaf parameters of mulberry saplings under nursery conditions

Parameters and Treatments	No of leaves per sapling		Leaf area (cm ²)		Fresh leaf weight per sampling (g)		Dry leaf weight per sampling (g)		Leaf moisture content (%)	
	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)
T ₀	27.86 ^c	22.33 ^c	166.05 ^c	149.81 ^c	87.25 ^c	68.53 ^c	24.54 ^c	18.50 ^c	71.87	73.00
T ₁	28.91 ^c	23.33 ^c	171.26 ^c	153.66 ^c	89.06 ^c	70.25 ^c	25.03 ^c	18.91 ^c	71.90	73.08
T ₂	32.95 ^a	26.89 ^a	187.91 ^a	165.66 ^a	96.11 ^a	76.43 ^a	26.72 ^a	20.23 ^a	72.20	73.53
T ₃	28.41 ^d	22.92 ^d	169.34 ^d	151.76 ^d	88.01 ^d	69.28 ^d	24.74 ^d	18.69 ^d	71.89	73.02
T ₄	30.02 ^b	24.19 ^b	176.36 ^b	156.94 ^b	90.05 ^b	71.14 ^b	25.28 ^b	19.11 ^b	71.93	73.14
C.D ($p \leq 0.05$)	0.108	0.206	0.884	0.791	0.318	0.195	0.096	0.044	NS	NS

* Each value is the Mean of three separate observations.

T₀ (control for each variety), T₁ (microclimate modification through smoke), T₂ (tender shoot covered with insulating bag/black polythene), T₃ (sapling covered with plastic bottle), and T₄ (split fertilizer application)

Table 4: Effect of frost protection techniques on shoot parameters of mulberry saplings under nursery conditions

Parameters and Treatments	Shoot length (cm)		Fresh shoot weight per sapling		Dry shoot weight per sapling (g)		Shoot Dia (cm)	
	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)
T ₀	100.19 ^c	84.79 ^c	149.96 ^c	114.06 ^c	59.04 ^c	46.72 ^c	1.07 ^c	1.03 ^c
T ₁	103.84 ^c	88.35 ^c	156.55 ^c	118.72 ^c	62.52 ^c	49.48 ^c	1.12 ^c	1.08 ^c
T ₂	118.25 ^c	100.94 ^c	174.74 ^c	133.38 ^c	69.16 ^c	55.22 ^c	1.34 ^a	1.28 ^a
T ₃	102.11 ^d	86.84 ^d	154.16 ^d	116.73 ^d	60.53 ^d	47.73 ^d	1.10 ^d	1.05 ^d
T ₄	107.85 ^b	91.42 ^b	162.52 ^b	122.66 ^b	63.64 ^b	50.47 ^b	1.18 ^b	1.12 ^b
C.D ($p \leq 0.05$)	0.701	0.658	0.196	0.095	0.067	0.080	0.017	0.017

* Each value is the Mean of three separate observations.

T₀ (control for each variety), T₁ (microclimate modification through smoke), T₂ (tender shoot covered with insulating bag/black polythene), T₃ (sapling covered with plastic bottle), and T₄ (split fertilizer application).

Table 5: Effect of frost protection techniques on root parameters of mulberry saplings under nursery conditions

Parameters and Treatments	No of roots per sapling		Fresh root weight per sapling (g)		Dry root weight per sapling (g)		Longest root length per sapling (cm)		Root volume per sapling (cm ³)	
	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)
T ₀	22.44 ^d	18.81 ^c	27.05 ^c	23.08 ^c	8.17 ^c	6.53 ^d	27.75	25.13	20.17 ^c	16.03 ^c
T ₁	22.75 ^c	19.08 ^c	27.21 ^c	23.24 ^c	8.29 ^c	6.67 ^c	27.91	25.22	20.29 ^c	16.12 ^c
T ₂	23.52 ^b	19.75 ^b	28.30 ^b	24.15 ^b	8.52 ^b	6.83 ^b	28.01	25.29	21.13 ^b	16.86 ^b
T ₃	22.52 ^c	18.89 ^c	27.13 ^c	23.18 ^c	8.24 ^c	6.61 ^c	27.84	25.18	20.22 ^c	16.07 ^c
T ₄	25.10 ^a	20.92 ^a	30.17 ^a	25.47 ^a	9.01 ^a	7.20 ^a	28.32	25.49	22.51 ^a	17.87 ^a
C.D ($p \leq 0.05$)	0.306	0.270	0.358	0.298	0.121	0.102	NS	NS	0.392	0.249

* Each value is the Mean of three separate observations.

T₀ (control for each variety), T₁ (microclimate modification through smoke), T₂ (tender shoot covered with insulating bag/black polythene), T₃ (sapling covered with plastic bottle), and T₄ (split fertilizer application)

Table 6: Effect of frost protection techniques on biochemical parameters of mulberry leaves under nursery conditions

Parameters and Treatments	Soluble protein (mg/g)		Total soluble sugars (mg/g)		Total phenols (mg/g)		Proline (μ mol/g)	
	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)
T ₀	22.20 ^c	23.79 ^c	33.12 ^c	35.23 ^c	0.201 ^c	0.319 ^c	27.72 ^c	29.12 ^c
T ₇	23.85 ^c	25.47 ^c	34.86 ^c	37.11 ^c	0.482 ^c	0.569 ^c	28.31 ^c	29.41 ^c
T ₉	24.65 ^b	26.28 ^b	35.57 ^b	37.88 ^b	0.651 ^b	0.743 ^b	28.73 ^b	30.25 ^b
T ₁₀	23.12 ^d	24.72 ^d	34.07 ^d	36.22 ^d	0.362 ^d	0.441 ^d	28.19 ^d	29.72 ^d
T ₁₁	26.23 ^a	27.90 ^a	37.21 ^a	39.62 ^a	0.999 ^a	1.145 ^a	29.62 ^a	31.23 ^a
C.D ($p \leq 0.05$)	0.016	0.018	0.022	0.023	0.033	0.027	0.025	0.021

* Each value is the Mean of three separate observations.

T₀ (control for each variety), T₁ (microclimate modification through smoke), T₂ (tender shoot covered with insulating bag/black polythene), T₃ (sapling covered with plastic bottle), and T₄ (split fertilizer application).

Table 7: Effect of frost protection techniques on Chlorophyll content of mulberry leaves under nursery conditions

Parameters and Treatments	Chlorophyll-a (mg/g)		Chlorophyll-b (mg/g)		Total chlorophyll (mg/g)	
	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)
T ₀	1.19 ^c	1.25 ^c	0.40 ^d	0.42 ^c	1.59 ^c	1.67 ^c
T ₇	1.25 ^c	1.37 ^c	0.43 ^c	0.46 ^c	1.68 ^c	1.83 ^c
T ₉	1.29 ^b	1.44 ^b	0.46 ^b	0.50 ^b	1.75 ^b	1.94 ^b
T ₁₀	1.22 ^d	1.36 ^d	0.42 ^c	0.44 ^d	1.64 ^d	1.79 ^d
T ₁₁	1.42 ^a	1.60 ^a	0.49 ^a	0.55 ^a	1.91 ^a	2.15 ^a
C.D ($p \leq 0.05$)	0.017	0.017	0.019	0.018	0.029	0.024

* Each value is the Mean of three separate observations.

T₀ (control for each variety), T₁ (microclimate modification through smoke), T₂ (tender shoot covered with insulating bag/black polythene), T₃ (sapling covered with plastic bottle), and T₄ (split fertilizer application)

Table 8: Effect of frost protection techniques on anti-oxidant potential of mulberry leaves under nursery conditions

Parameters and Treatments	DPPH (mg TE/g)		FRAP (mg TE/g)		SOD (U/mg or units/mg)		PPO (AA435/min/g)		Catalase (U/g or units/gram)	
	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)	V1 (Goshoerami)	V2 (Kokuso-21)
T ₀	14.11 ^c	15.45 ^c	19.96 ^c	21.07 ^c	160.81 ^c	162.62 ^c	1.31 ^c	1.58 ^c	187.96 ^c	189.14 ^c
T ₇	14.58 ^c	15.99 ^c	21.82 ^c	23.11 ^c	163.22 ^c	165.24 ^c	1.40 ^c	1.70 ^c	190.39 ^c	191.78 ^c
T ₉	15.17 ^b	16.62 ^b	22.14 ^b	23.47 ^b	164.34 ^b	166.37 ^b	1.44 ^b	1.76 ^b	191.67 ^b	193.18 ^b
T ₁₀	14.43 ^d	15.81 ^d	21.52 ^d	22.75 ^d	162.13 ^d	164.13 ^d	1.35 ^d	1.63 ^d	189.18 ^d	190.46 ^d
T ₁₁	16.31 ^a	17.83 ^a	22.66 ^a	24.03 ^a	166.76 ^a	169.42 ^a	1.51 ^a	1.85 ^a	194.52 ^a	196.28 ^a
C.D ($p \leq 0.05$)	0.020	0.021	0.023	0.024	0.206	0.333	0.017	0.017	0.983	0.852

* Each value is the Mean of three separate observations.

T₀ (control for each variety), T₁ (microclimate modification through smoke), T₂ (tender shoot covered with insulating bag/black polythene), T₃ (sapling covered with plastic bottle), and T₄ (split fertilizer application)

Conclusion

Polythene shoot covering (T₂) proved most effective in reducing frost damage and promoting vigorous spring growth in mulberry saplings. Split fertilizer application (T₄) enhanced root traits and triggered strong biochemical and antioxidant responses, improving internal cold tolerance. Overall, combining physical protection with balanced nutrient management offers a reliable strategy for reducing frost injury and supporting healthy early growth of mulberry under temperate nursery conditions for sustainable sericulture.

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