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Mulberry leaf quality influenced by surface, subsurface manuring and fertilizer application in irrigated conditions of Karnataka

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Abstract
A field experiment was conducted for three crop cuttings of mulberry (2023-2024) in Kurburu village of Chinthamani taluk to test the effect of surface, subsurface manuring and fertilizer application on leaf quality of mulberry crop in irrigated condition. The experiment was laid out in split plot design with fourteen treatment combinations and three replications. Main plots include two different types of manuring *viz*., Surface manuring M_1 and Subsurface manuring-M_2 at 60-75 cm depth with FYM (Farm yard manure) and enriched manure. Subplots include R_1-Absolute control, R_2-50% RDF + FYM, R_3-75% RDF + FYM, R_4-100% RDF + FYM, R_5-50% RDF + enriched compost, R_6-75% RDF + enriched compost, R_7-100% RDF + enriched compost, quality parameters leaf area, leaf area index and chlorophyll content were recorded at 30, 45 and 60 Days after pruning (DAP) for three crops. The protein content was recorded at harvest of mulberry. The results of the experiment revealed that the subsurface manuring with enriched manure recorded highest single leaf area (110.19, 156.73 and 235.90 cm^2), LAI (4.53, 6.44 and 9.69), chlorophyll SPAD reading (54.53, 54.99 and 54.90), protein content (29.38, 29.64 and 31.06%) for first, second and third crops. Effect of subsurface manuring is differed from surface manuring due to direct availability of nutrients at rhizosphere zone enhance uptake of nutrients like nitrogen helps to increase leaf area, LAI, chlorophyll SPAD reading and protein content.

Keywords: Chlorophyll content, leaf area, quality, surface manure, subsurface manure, fertilization

Introduction
The quality of mulberry leaves is a critical factor in providing essential nourishment to silkworms, directly impacting their development and the synthesis of silk proteins, which are vital for silk production. Mulberry silk quality is influenced by soil fertility and environmental conditions, spotlight the need to thoroughly understand these factors to optimize mulberry cultivation (Rashmi *et al.*, 2009) [19]. Intensive cropping practices involving regular harvesting of mulberry leaf shoot biomass (five harvests per year, yielding approximately 80-100 metric tons per hectare annually), significantly deplete soil nutrients. To improve quality and dry matter production needs regular supplementation of essential nutrients and soil conditioning through organic manuring are crucial for maintaining soil fertility and producing high-quality mulberry leaves (Shashidhar *et al.*, 2009) [20]. Balanced fertilization has been shown to enhance leaf quality and increase silk protein production in silkworms, leading to improved cocoon yields. Furthermore, factors such as mulberry genotype, cultivation practices, soil moisture and the nutrient composition of garden soils play a significant role in determining the yield and quality of mulberry leaves (Chen *et al.* 2009) [3] and (Singh *et al.* 2014) [22]. Mulberry prefers a soil with almost neutral pH for its lush growth (Rangaswami *et al.*, 1976) [18]. In light of the apparent decline in soil fertility deliberate efforts are necessary to promote the careful application of organic manures in order to achieve optimal leaf yield in mulberry. The soil and water are the most essential natural resources in *rainfed* sericulture. Dry lands typically have low fertility and struggle to retain moisture. Owing to the erratic rainfall crops in *rainfed* areas experience high water evaporation and low water retention in the soil.

The amount of manure and chemical fertilizers recommended for mulberry cultivation is relatively high compared to other agricultural crops (Hanumanta, 2024) [7]. Excessive use of chemicals and improper selection of fertilizers in mulberry cultivation causing great concern for the survivability of soil fauna and flora as well as soil health. The use of synthetic fertilizers has been demonstrated to directly impact the makeup of the soil's microbial community and its functional diversity (Yu *et al.*, 2015) [24]. However, the impact of inorganic fertilizer on soil properties particularly soil microbial abundance community structure and functional diversity is not well understood in mulberry plantations. Conflicting and uncertainties remain regarding the long-term effects of inorganic fertilizers on the soil microbial community and functional diversity. To sustain quality mulberry leaf production, farmers should replenish the soil nutrients by applying the recommended quantity of NPK fertilizers through 140 kg of ammonium sulfate, 70 kg of single superphosphate and 18 kg of muriate of potash per acre per crop (Dandin *et al.*, 2014) [4].

Mulberry leaf is rich in essential amino acids, especially lysine (1.80%) and leucine (2.58%). It contains 8.01-13.42 g of carbohydrates, 4.72-9.96 g of crude protein, 0.64-1.51% crude fat, 4.26-5.32 g of total ash, and provides 69-86 kcal/100 g of energy (Banerjee *et al.*, 2016) [1]. Despite its nutritional significance, limited studies have examined the biochemical composition of different mulberry parts. This study focuses on evaluating the quality parameters of mulberry leaves.

Material and Methods

Site description

The study site located at Kurburu village, Chintamani taluk, Chikkaballapur district, Karnataka. The experimental field is located at a latitude of 13º312 N and longitude of 78º.071 East and at an altitude of 930 m above mean sea level in the eastern dry zone of Karnataka (Zone-5).

In situ experiment details

The experiment consists of two main plots and seven subplots like Surface manuring M_1 and Subsurface manuring-M_2 at 60-75 cm depth with FYM (Farm yard manure) and enriched manure. Subplots include R_1-Absolute control, R_2-50% RDF + FYM, R_3-75% RDF + FYM, R_4-100% RDF + FYM, R_5-50% RDF + enriched compost, R_6-75% RDF + enriched compost, R_7-100% RDF + enriched compost. The recommended dose of fertilizers (NPK)-350:140:140 kg per hectare per year, FYM-25 tonnes per hectare per year and enriched compost prepared by adding single super phosphate and microbial consortia. The subsurface manure applied for first crop (June 2023) and next two were residual crops. The interaction of main plot and subplots resulted fourteen treatments with three replications in split plot design were observed.

Sampling

The leaf area of the third fully expanded leaf from the top was measured using a leaf area meter with data collected from five plants per treatment across replicates on the 30, 45 and 60th days after pruning. The average leaf area was then calculated. Leaf area index (LAI) was measured by using CL-110 Plant canopy Digital imager instrument. Chlorophyll content in mulberry leaves was measured using a SPAD (Soil Plant Analysis Development) meter. The average chlorophyll content was recorded for the top, middle and bottom leaves of five randomly selected plants and the results were presented. The protein content in the leaf sample was determined by plotting a standard absorbance curve with absorbance at 660 nm (A660) on the Y-axis and protein concentration on the X-axis. The total protein content was then expressed as a percentage.

Statistical analysis

The data on leaf quality parameters of mulberry plant were recorded at 30th, 45th and 60th DAP in each treatment on randomly selected five plants from each replication and mean value of three crops were calculated. The experimental data collected on leaf quality components of plant were subjected to Fisher's method of Analysis of Variance (ANOVA) as outlined by Panse and Sukhatme (1967) [15].

Results and Discussion

Leaf Area (cm^2)

The findings on leaf area (cm^2) were significantly influenced by place of manure application and combination of manure applied at 30, 45 and 60 DAP (Table 1) and leaf area varied significantly due to different treatments of mulberry crop. The highest leaf area (109.36, 110.56 and 110.67 cm^2) was recorded in treatment M_2 which received subsurface manuring than M_1 which received surface manuring having lowest leaf area (108.56, 109.75 and 109.85 cm^2) was observed in the first, second and third crops respectively at 30 DAP. Higher leaf area (157.29, 156.35 and 156.54 cm^2) was observed in treatment with subsurface manuring whereas lower leaf area was observed in treatment with surface manuring (155.62, 156.19 and 156.34 cm^2) in first, second and third crops respectively at 45 DAP. Higher leaf area (235.53, 236.01 and 236.15 cm^2) was observed in treatment with subsurface manuring whereas lower leaf area was observed in treatment with surface manuring (234.00, 234.29 and 234.25 cm^2) in first, second and third crops respectively at 60 DAP. Subsurface manuring has proven to be an efficient manuring method with potential advantages of high nutrient use efficiency. These results might be due to nitrogen, phosphorus and potassium presence in manure and fertilizers, which is also a major osmotically active component in plant cells contributing to cell turgor and enhancing the capacity of plant cell to retain water and nutrients, decreased water loss from plants by transpiration and water use efficiency and nutrient use efficiency were increased and hence there is also improvement in growth characters. Thus application of manure with fertilizers can promote plant growth with better quality. The study is also supported by Mir *et al.* (2010) [13] reported that an improvement in various growth parameters when different mulches were applied in mulberry under temperate conditions leads increase in leaf quality. The findings of Gangwar *et al.* (2000) [6]; Purohit *et al.* (2006) [16] and Dilip (2007) [5] in mulberry, Kumar *et al.* (2018) [11] in strawberry and Chandra and Govind (2001) [2] in ginger.

Among fertilizer recommendation, leaf area was significantly influenced by different fertilizer recommendations in mulberry during three crops. 100% RDF with enriched compost in mulberry plot has recorded significantly highest leaf area (119.76, 121.41 and 121.54 cm^2), which was followed by R_6: 75% RDF + enriched compost plots (116.91, 117.92 and 118.17 cm^2) and plot with R_5: 50% RDF + enriched compost (113.18, 111.50 and 111.66 cm^2), among fertilizer recommendation lowest was found in R_2: 50% RDF + FYM treated plots (102.59, 105.67 and 105.77 cm^2) and in R_1: Absolute control plots without fertilizer recommendation (95.52, 97.48 and 97.40 cm^2) respectively. However, third crop has recorded highest leaf area compared to first two crops with 100% RDF + enriched compost in mulberry plot.

Table 1: Mulberry leaf area as influenced by surface and subsurface manuring and fertilizer application at 30, 45 and 60 DAP

Methods of application (M) Treatments	Single leaf area (cm^2)											
	I Crop			II Crop			III Crop			Pooled data		
	30 DAP	45 DAP	60 DAP	30 DAP	45 DAP	60 DAP	30 DAP	45 DAP	60 DAP	30 DAP	45 DAP	60 DAP
M_1: Surface manuring	108.56	155.62	234.00	109.75	156.19	234.29	109.85	156.34	234.25	109.39	156.05	234.18
M_2: Subsurface manuring	109.36	157.29	235.53	110.56	156.35	236.01	110.67	156.54	236.15	110.19	156.73	235.90
S.Em.±	-	-	-	0.05	-	-	0.02	-	-	0.004	-	0.07
CD ($p\leq0.05$)	-	-	-	0.33	-	-	0.13	-	-	0.03	-	0.46
F-test	NS	NS	NS	*	NS	NS	*	NS	NS	*	NS	*
Fertilizer recommendation (R)												
R_1: Absolute control	95.52	141.39	207.74	97.48	141.52	208.11	97.40	141.90	208.38	96.80	141.60	208.08
R_2: 50% RDF + FYM	102.59	145.32	227.16	105.67	145.80	228.15	105.77	145.85	228.35	104.68	145.66	227.89
R_3: 75% RDF + FYM	106.04	147.74	234.12	107.41	149.14	234.27	107.70	149.23	234.33	107.05	148.70	234.24
R_4: 100% RDF + FYM	108.75	154.39	238.43	109.67	154.75	239.37	109.56	154.94	239.45	109.33	154.69	239.09
R_5: 50% RDF + enriched compost	113.18	166.01	240.26	111.50	164.99	239.36	111.66	165.06	239.41	112.11	165.35	239.68
R_6: 75% RDF + enriched compost	116.91	169.07	247.42	117.92	167.28	247.86	118.17	167.49	247.50	117.67	167.95	247.59
R_7: 100% RDF + enriched compost	119.76	171.27	248.22	121.41	170.41	248.94	121.54	170.64	248.97	120.90	170.77	248.71
S.Em.±	0.74	0.93	1.77	0.74	1.29	1.79	0.74	1.26	1.83	0.42	0.36	0.19
CD ($p\leq0.05$)	2.15	2.71	5.17	2.16	3.76	5.21	2.17	3.69	5.34	1.24	1.06	0.56
F-test	*	*	*	*	*	*	*	*	*	*	*	*
Interaction (M×R)												
T_1-M_1 R_1	95.83	141.07	207.34	97.41	141.36	208.04	97.45	141.40	208.18	96.90	141.28	207.85
T_2-M_1 R_2	102.97	145.07	225.81	105.38	145.50	226.54	105.41	145.57	226.56	104.59	145.38	226.30
T_3-M_1 R_3	105.88	147.28	232.74	107.02	148.21	232.98	107.24	148.29	233.01	106.71	147.93	232.91
T_4-M_1 R_4	108.84	154.01	237.70	109.53	154.56	238.18	109.58	154.87	238.27	109.32	154.48	238.05
T_5-M_1 R_5	109.78	164.34	240.30	110.46	164.96	238.58	110.52	165.05	238.66	110.25	164.78	239.18
T_6-M_1 R_6	116.74	167.79	246.64	117.71	168.56	247.22	117.84	168.62	246.49	117.43	168.32	246.78
T_7-M_1 R_7	119.91	169.76	247.45	120.71	170.18	248.51	120.89	170.59	248.56	120.50	170.18	248.17
T_8-M_2 R_1	95.21	141.71	208.14	97.55	141.67	208.18	97.34	142.39	208.58	96.70	141.92	208.30
T_9-M_2 R_2	102.21	145.56	228.51	105.97	146.10	229.76	106.12	146.13	230.14	104.77	145.93	229.47
T_{10}-M_2 R_3	106.20	148.20	235.51	107.80	150.08	235.56	108.16	150.16	235.64	107.39	149.48	235.57
T_{11}-M_2 R_4	108.65	154.77	239.16	109.81	154.94	240.57	109.54	155.00	240.63	109.33	154.90	240.12
T_{12}-M_2 R_5	116.58	167.68	240.22	112.53	165.02	240.14	112.80	165.07	240.16	113.97	165.92	240.17
T_{13}-M_2 R_6	117.08	170.34	248.20	118.13	166.00	248.50	118.50	166.37	248.51	117.90	167.57	248.40
T_{14}-M_2 R_7	119.60	172.78	248.98	122.10	170.63	249.36	122.19	170.68	249.38	121.30	171.36	249.24
S.Em.±	1.04	-	-	-	-	-	-	-	-	-	-	0.26
CD ($p\leq0.05$)	3.03	-	-	-	-	-	-	-	-	-	-	0.75
F-test	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*

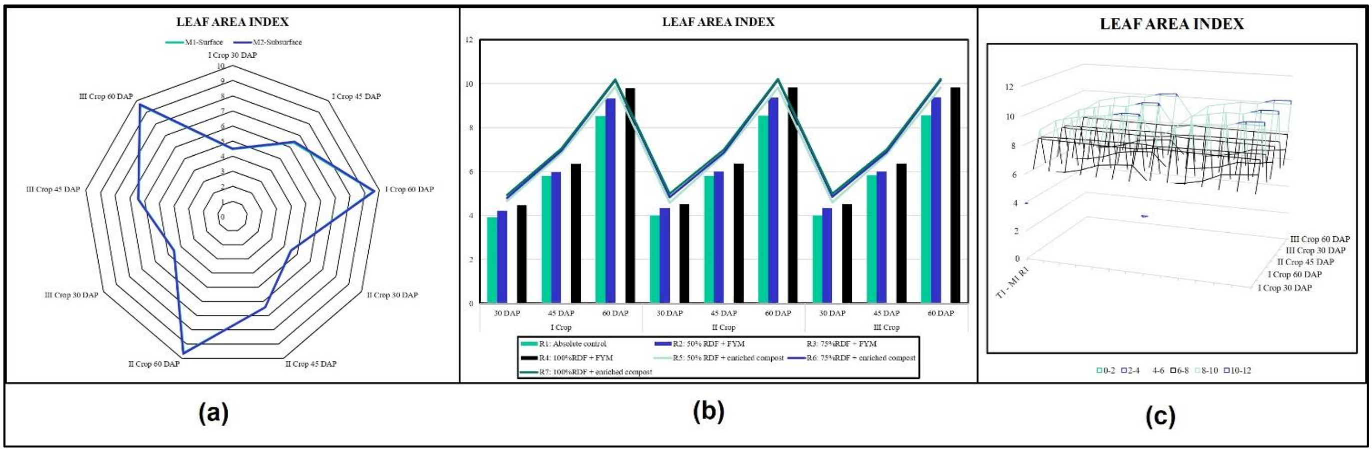
Note: FYM-Farm yard manure, Recommended dose of NPK-350:140:140 kg ha^{-1} yr^{-1}, DAP-Days after pruning *-Significant, NS-Non significant

Leaf area in mulberry plant was significantly influenced due to the interaction effect of surface, subsurface manuring and fertilization in mulberry during three crop growths at 30 DAP. However, T_{14}-M_2R_7 which is subsurface manuring with 100% RDF + enriched compost recorded highest leaf area (119.60, 122.10 and 122.19 cm^2) and lowest was recorded in T_1-M_1R_1 which is control without manure (95.53, 97.41 and 97.45 cm^2) during first, second and third crops respectively. This might be due to the activity of microorganisms increase nutrient availability it helps to increase in photosynthesis. Similarly, Ramya *et al.* 2023 [17] observed the leaf area (cm^2) significantly influenced by types of irrigation and organic mulches at 30, 45 and 60 DAP and leaf area varied significantly due to different treatments of mulberry crop. Subsurface drip irrigation @ 0.75 CPE increased leaf area (115.45, 165.31 and 241.73 cm^2 respectively) varied significantly over surface drip irrigation @ 0.75 CPE (108.03, 149.29 and 223.52 cm^2 respectively).

Leaf area index

The results on leaf area index were significantly influenced by place of manure application and combination of manure applied at 30, 45 and 60 DAP (Fig. 1: a, b and c). The leaf area index (LAI) of mulberry varied significantly across treatments. Subsurface manuring (M2) consistently resulted in higher LAI than surface manuring (M1). At 30 DAP, LAI was higher in M2 (4.49-4.54) compared to M1 (4.46-4.51). Similarly, at 45 DAP, subsurface manuring recorded LAI values of 6.42-6.46, while surface manuring had 6.39-6.42. At 60 DAP, LAI was highest in subsurface manuring (9.67-9.70) and lowest in surface manuring (9.61-9.62), indicating the effectiveness of subsurface manure application in improving mulberry growth. Among fertilizer recommendation, leaf area index was significantly influenced by different fertilizer recommendations in mulberry during three crops. 100% RDF with enriched compost in mulberry plot has recorded significantly highest leaf area index (10.20, 10.22 and 10.22), which was followed by R_6: 75% RDF + enriched compost plots (10.16, 10.18 and 10.16) and in R_1: Absolute control plots without fertilizer recommendation (8.53, 8.55 and 8.56) respectively. However, third crop has recorded highest leaf area index compared to first two crops. This might be due to the increase in growth parameters, such as the number of leaves per branch, number of leaves per plant with the significant impact of subsoil manuring with enriched organic manure and mineral fertilizers on the growth attributes of the mulberry crop. This effect can be attributed to the enhancement of soil physical properties achieved through subsoil manuring, which promotes root development and facilitates improved water and nutrient uptake during crop growth stages.

Consequently, these factors contribute to an overall increase in crop yield. Similar results for application of organic manures was obtained by Subbarao and Ravisankar (2001) [23]; Kannan *et al.* (2013) [9] attributed the results to the balanced and sustained release of available nitrogen from manure through organic matter mineralization. Light interception, dry matter production, accumulation and partitioning are all improved as a result of it. Because higher dry matter production is an important pre-requisite for higher crop yields as it signifies the photosynthetic ability of the crop and also indicates other biosynthetic processes during development (Shashikumar, 2002) [21].



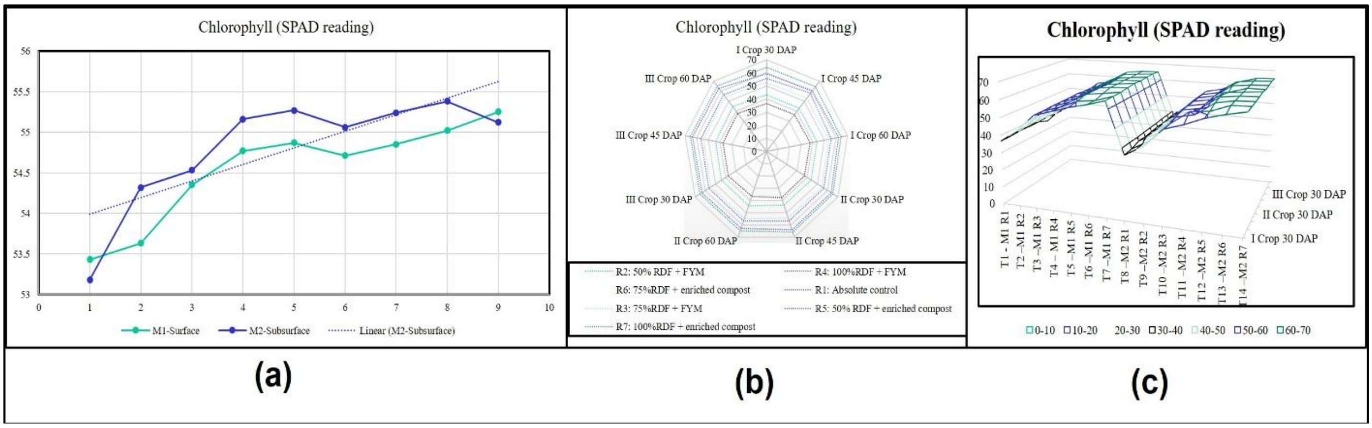
*FYM-Farm yard manure; RDF-Recommended dose of fertilizers; DAP-Days after pruning

Fig. 1: (a) Effect of surface and subsurface manuring, (b) Effect of different levels of fertilization and manures, (c) Interaction effect of surface and subsurface manuring and fertilization on leaf area index

Chlorophyll content (SPAD reading)

The findings on chlorophyll content (SPAD reading) were significantly influenced by place of manure application and combination of manure applied at 30, 45 and 60 DAP (Fig. 2a, 2b and 2c) and chlorophyll content varied significantly due to different treatments of mulberry crop. The highest leaf chlorophyll content (55.16 and 55.24) was recorded in treatment M_2 which received subsurface manuring than M_1 which received surface manuring having lowest leaf chlorophyll content (54.77 and 54.85) was observed in the second and third crops respectively at 30 DAP. Higher leaf chlorophyll content (54.32, 55.27 and 55.38) was observed in treatment with subsurface manuring whereas lower leaf chlorophyll content was observed in treatment with surface manuring (53.63, 54.87 and 55.02) in first, second and third crops respectively at 45 DAP. Higher leaf chlorophyll content (54.53 and 55.06) was observed in treatment with subsurface manuring whereas lower leaf chlorophyll content was observed in treatment with surface manuring (54.35 and 54.71) in first, second crops respectively at 60 DAP. Subsurface manuring has proven to be an efficient manuring method with potential advantages of high nutrient use efficiency. The CM 1000 chlorophyll meter reading of 745.33 is equal to 87.5 units of SPAD reading (Mahajan *et al.*, 2014) [12]. Jangir *et al.* (2021) [8] reported that residual effect of organic treatment (50 per cent RDN through FYM and 50 per cent RDN through biocompost) and integrated nutrient treatment (50 per cent RDN through FYM and 50 per cent RDN through chemical fertilizer or 50 per cent RDN through biocompost and 50 per cent RDN through chemical fertilizer) have given higher chlorophyll content in leaves of cowpea over chemical fertilizer alone. This could be attributed to the ability of organic fertilizers, as well as their combination with chemical fertilizers, to supply plants with a higher amount of residual nitrogen. This is due to the slow release of nitrogen from complex compounds and reduced nitrogen losses through leaching.

Subsurface manuring with 100% RDF + enriched compost recorded highest leaf chlorophyll content (64.81, 64.77 and 65.10) and lowest was recorded in T_1-M_1R_1 which is control without manure (37.44, 35.09 and 37.83) during first, second and third crops respectively. Surface, subsurface manuring and fertilization promotes the development of a healthy root system which in turn supports better nutrient uptake and growth parameters like number of leaves per plant, leaf area, leaf area index, leaf chlorophyll content. Similarly, Ondieki *et al.* (2011) [14] found higher chlorophyll a and b in organically amended plots than in non amended control in *Solanum nigrum*. This might be due to the decomposition of organic inputs releases various nutrients that enhance protein synthesis, amino acid production and photosynthetic activity. This leads to better root development and ultimately increases potassium content, which plays a crucial role in stomatal regulation. These observations are validating the findings of the current study.

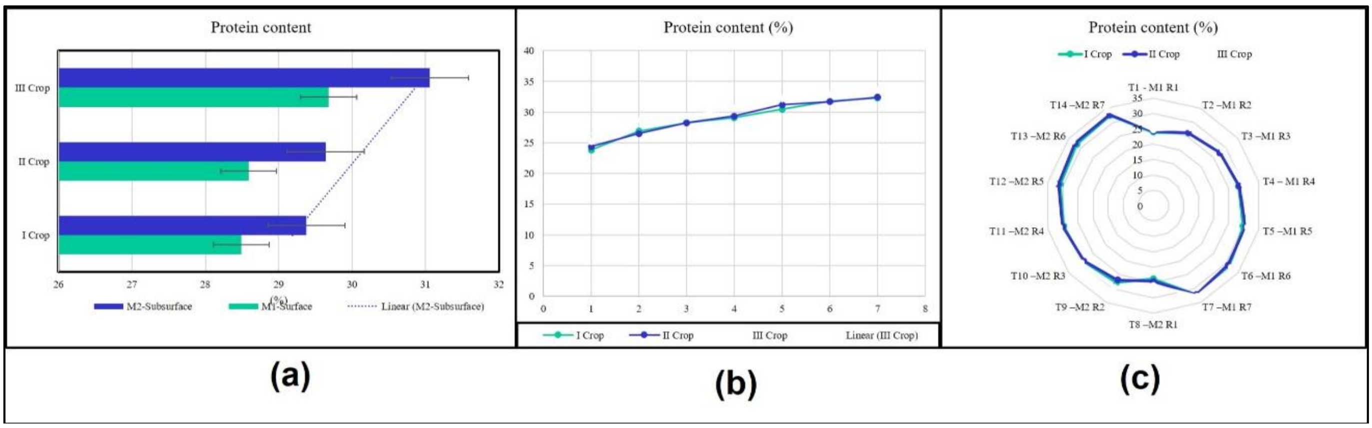


*FYM-Farm yard manure; RDF-Recommended dose of fertilizers; DAP-Days after pruning

Fig 2: (a) Effect of surface and subsurface manuring, (b) Effect of different levels of fertilization and manures, (c) Interaction effect of surface and subsurface manuring and fertilization on chlorophyll content (SPAD reading)

Protein content (%)

The results on Protein content were significantly influenced by place of manure application and combination of manure applied at 60 DAP (Fig. 3a, 3b and 3c). The data on Protein content varied significantly due to different treatments of mulberry crop. Higher leaf protein content (29.38, 29.64 and 31.06%) was observed in treatment with subsurface manuring whereas lower leaf protein content was observed in treatment with surface manuring (28.49, 28.59 and 29.68%) in first, second and third crops respectively. Among fertilizer recommendation, leaf protein content was significantly influenced by different fertilizer recommendations in mulberry during three crops. 100% RDF with enriched compost in mulberry plot has recorded significantly highest leaf protein content (32.30, 32.44 and 33.53%), which was followed by R_6: 75% RDF + enriched compost plots (31.76, 31.72 and 32.90%) and in R_1: Absolute control plots without fertilizer recommendation (23.80, 24.39 and 25.21%) in first, second and third crops respectively. Leaf protein content in mulberry differed significantly in pooled data of three crops due to the interaction effect of methods of surface, subsurface manuring and fertilization. However, T_{14}-M_2R_7 which is subsurface manuring with 100% RDF + enriched compost recorded highest leaf protein content (32.51, 32.82 and 34.40%) and lowest was recorded in T_1-M_1R_1 which is control without manure (23.81, 24.17 and 24.53%). The protein improvement could be due to enhancement of nitrogen concentration with combined and sole application of compost and chemical fertilizer. Similar results reported by Kemal *et al.* (2018) [10], that the protein content was marginally higher in 2015 than in 2016 due to a greater nitrogen concentration in the seeds during the first season compared to the second. In comparison to the unfertilized treatment, the application of 100% RDF, 6 tons of compost and the combined application of 6 tons of compost with 75%, 50%, and 25% RDF resulted in increases in seed protein content by 63%, 23%, 61%, 69% and 60%, respectively.



*FYM-Farm yard manure; RDF-Recommended dose of fertilizers; DAP-Days after pruning

Fig 3: (a) Effect of surface and subsurface manuring, (b) Effect of different levels of fertilization and manures, (c) Interaction effect of surface and subsurface manuring and fertilization on protein content

Conclusion

The study demonstrated that the placement of appropriate quantity of manure application significantly influenced mulberry leaf quality parameters. Subsurface manuring proved to be an efficient method, enhancing macro and micro nutrient use efficiency and resulting in higher single leaf area, LAI (leaf area index), leaf chlorophyll content and protein content compared to surface manuring. The highest single leaf area, T_{14}-M_2R_7 which is subsurface manuring with 100% RDF + enriched compost (119.60, 122.10 and 122.19 cm^2), LAI (10.20, 10.22 and 10.22), leaf chlorophyll content (64.81, 64.77 and 65.10), highest leaf protein content (32.51, 32.82 and 34.40%) in first, second and third crops respectively in subsurface-manured plots compared to surface-manured treatments. Therefore, this study suggests that the subsurface application of enriched manure + RDF enhances mulberry leaf growth, quality and protein content by reducing nutrient losses while maximizing nitrogen use efficiency.

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