



Effect of Weed Management Approaches on Leaf Nutrient and Chlorophyll Content in Apple (*Malus x domestica* Borkh.) Nursery Plants Raised on Clonal Rootstock

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AUH, AA, AK, FAK, SAM and BAA designed the study. Authors AUH and AA managed the literature searches. Authors AUH, AA and AK managed experimental materials and data collection. Authors AUH, FF and FMM wrote the first draft of the manuscript. All authors read and approved the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The present study aimed to determine the effects of various weed management approaches on the leaf nutrient and chlorophyll content in apple nursery plants raised on clonal rootstock M9-T337. Weed management approaches evaluated consisted of seven treatments viz; manual weeding, pendimethalin @ 1 kg a.i. ha⁻¹, pendimethalin @ 1 kg a.i. ha⁻¹ + manual weeding, paddy straw mulch-6 cm thick, black polyethylene mulch-200 micron, weed-free and weedy check laid out in randomized complete block design and replicated thrice. Data on the leaf nutrients (Nitrogen, phosphorus and potassium) and chlorophyll content in leaves were recorded. Weed control treatments significantly influenced the leaf nutrient status of apple nursery plants. Significantly higher content of leaf nitrogen, phosphorus and potassium as well as chlorophyll content were recorded with paddy straw mulch but were at par with black polyethylene mulch treatment. In conclusion, weed management through paddy straw mulch was found to be beneficial for higher leaf nutrient and chlorophyll content in grafted apple nursery plants.

Keywords: Apple Nursery; leaf nutrient; chlorophyll; mulch; pendimethalin; weed control.

1. INTRODUCTION

Nutritional balance and biological activity are the primary determinants of plant growth and development. Plants require substantial amounts of nitrogen, phosphorus and potassium among the basic nutritional elements. Several important compounds in plants contain nitrogen (N), including proteins, amino acids, amides, nucleic acids, nucleotides, coenzymes, chlorophyll, cytosine and auxin [1-3]. Several authors have demonstrated a positive relationship between chlorophyll and N concentration in the leaves hence, chlorophyll content can be used as an alternative indicator of plant N status [4-6]. Phosphorus is a crucial component of nucleic acid that helps the plant to establish itself quickly after transplantation by stimulating root formation [7]. Stunted growth, short stems, and a dark green hue on the upper surface of the leaves with purpling veins are the symptoms of P deficiency. Potassium is another essential component for plant growth and development [8]. Potassium has a direct role in number of plant activities viz; photosynthesis, stomatal control and transpiration and abiotic stress [9], indicating the significance of appropriate potassium levels in plants for proper growth and development.

The apple (*Malus x domestica* Borkh.) is the world's most widely cultivated temperate fruit crop [10], with commercial plantings in China, the United States, Turkey, Poland, India, Italy, Iran, Russia, France, Chile, Brazil, Ukraine, and other temperate temperature locations [11]. Commercial apple production in India is mostly limited to regions of Jammu and Kashmir, Himachal Pradesh, Uttarakhand, although North Eastern viz; Arunachal Pradesh, Nagaland, and

Sikkim also producing apples on limited scale [12]. For the success of apple farming, it is critical to use high-quality planting material. Apples commercially propagated by vegetative means, such as grafting and budding of desired cultivars onto rootstocks. Clonal rootstocks are required in grafted plants in order to achieve high density planting.

Selection of suitable agronomic practices viz; propagation material, irrigation, nutrients, hoeing, and weeding, as well as pest and disease management are crucial in the production of high-quality grafted nursery plants. Although soil quality, crop traits and growing environment all impact plant nutrient uptake, appropriate weed control is critical for soil management and much more so for nursery plant growth and development. Weeds play significant role on partition of applied nutrients, resulting in a lower yield and deterioration of the situation when these resources become scarce [13]. Hence, effective weed control practices are required for optimal leaf nutritional status and chlorophyll content, which favours plant growth and development. The aim of present study was to evaluate the effect of different weed control approaches on the leaf nutrient and chlorophyll content in grafted apple plants in the nursery.

2. MATERIALS AND METHODS

2.1 Experimental Site

During the nursery raising season of 2020, the experiment was conducted at the Experimental Field of the Division of Fruit Science, Sher-e-Kashmir University of Agricultural Science and Technology of Kashmir (SKUAST-K), Shalimar Campus, Srinagar, Jammu and Kashmir (India).

The experimental site is located at 34.1° North latitude and 74.9° East longitude, with a height of 1587 metres above sea level.

2.2 Plant Materials

Plant material used for the weed management trial at the apple nursery in this study was Apple cv. Silver Spur grafted on M9-T337 rootstock. Silver Spur is a compact to medium tree with excellent spur density, medium and large size conical fruits with pronounced lobes, deep red skin with stripes, mature in the middle of the season, and is recommended for cultivation in Jammu & Kashmir, Himachal Pradesh, Arunachal Pradesh, and Uttarakhand [14]. M9-T337 is a dwarfing type clonal rootstock imported from Holland and used to produce grafted apple planting materials for high density planting in Kashmir. M9-T337 rootstock has morphologically weak plant vigour, with short internodes, reddish brown colour (sunny side), strong undulated leaf margin, and weak prudence on lower side of leaf, long petiole length, and small stipule [15].

2.3 Weed Control Treatments

Manual weeding (T_1), pendimethalin (T_2), pendimethalin + manual weeding (T_3), paddy straw mulch (T_4), black polyethylene mulch (T_5), weed free (T_6), and weedy check (T_7) were seven weed control treatments (T_7). To thoroughly cover the soil in each treatment plot, paddy straw mulch (6 cm thick) and black polyethylene mulch (200 micron) were spread on ground around the plants. Pendimethalin @ 1 kg a.i. ha¹ was applied as a pre-emergence herbicide in the treatment plots (T_2 and T_3) on March 15, 2020.

2.4 Experimental Design

The experiment was laid out in Randomized Complete Block Design and replicated thrice.

2.5 Data Collection

The following procedures were used to quantify the leaf nitrogen, phosphorus, potassium, and chlorophyll content of grafted apple plants in the nursery:

2.5.1 Nitrogen

The nitrogen content in leaves was determined by modified Kjeldhals method as outlined by

Jackson [16]. Reagents used for nitrogen determination were concentrated sulphuric acid, Sulphate mixture (K_2SO_4 , $FeSO_4$, $CuSO_4$ and selenium powder in 10, 3, 1 and 1 parts, respectively), standard sulphuric acid (N/50), indicator (a solution of bromocresol green 99 mg and methyl red 66 mg in 100 ml of ethanol), boric acid (4%) and sodium hydroxide (40%). For digestion of sample, plant sample (1 g) was loaded in a digestion tube of preheated digestion block and 12 ml of concentrated sulphuric acid and 10 g of digestion mixture was added to the digestion sample. The digestion was continued until the sample was clear with a blue-green solution. After the digestion was finished, the digest was cooled and diluted to a level of 100 ml. For nitrogen distillation, ten ml of boric acid indicator solution (4%) was poured in a conical flask and placed in the distillation unit, followed by thirty ml of NaOH (40%) dispensed into the tube, and the boric acid was titrated against a N/50 H_2SO_4 solution. The end point was determined by the colour change of the solution from green to a faint pink and thus the end point reading was recorded in all the sample solutions. Blank sample was also carried out to the same conclusion as performed in case of leaf samples.

2.5.2 Phosphorus

The Spectrophotometer was used to assess the phosphorus content in leaves digested leaf samples using the Vandate molybdate yellow colour technique [16]. The Reagents used for the purpose were Ammonium molybdate-ammonium vandate in HNO_3 [ammonium molybdate (22.5 g in 400 ml distilled water), ammonium vanadate (1.25g) in 300 ml boiling distilled water followed by addition of vanadate solution to the molybdate solution and cooling at room temperature and finally addition of concentrated HNO_3 (250 ml) and making the volume 1 litre], Phosphate standard solution (50 ppm), and Di-acid mixture [concentrated HNO_3 (9 parts) and distilled concentrated $HClO_4$ (4 parts)]. For digestion of the sample, the plant sample (1 g) was taken in conical flask (150 ml capacity) and diacid mixture (10 ml) was added to the sample, thereafter the sample in flask was heated on a hot plate until sample cleared. The digested plant sample was transferred to volumetric flask (100 ml capacity) and volume made to the capacity of flask with distilled water up to the mark.

The standard solutions of phosphorus having 0, 1, 2, 4, 5 and 6 ppm concentration were

prepared in 50 ml volumetric flasks for finding out the standard curve. Thereafter, vanodomolybdate reagent (10 ml) was added to each standard solution and final volume was made up of 50 ml. After 30 minutes, the absorbance of solution was measured with spectrophotometer at 420 nm and the absorbance was plotted against respective concentration of phosphorus. Five ml aliquat was taken in a 50 ml volumetric flask from the digested plant sample that has been previously made up to 100 ml. Ten ml of vandomolybdate reagent was added to aliquat and the volume was made up 50 ml by adding distilled water. The absorbance of final aliquat solution was measured with spectrophotometer at 420 nm and phosphorus concentration was worked out with the help of standard curve.

2.5.3 Potassium

Potassium content in leaves under each treatment was determined using flame photometer [16]. Reagents used for potassium determination was standard stock solutions of potassium (1000 ppm in distilled water). The Standard solutions of potassium at 0, 20, 40, 60, 80 and 100 ppm were prepared for finding out the standard curve of the potassium. Plant sample (1 g) was taken in the conical flask of 150 ml capacity and diacid mixture (10 ml) was added to the sample and then it was heated on a hot plate until sample cleared. Leaf digest was transferred in volumetric flask (100 ml capacity) and the volume made to 100 ml by adding distilled water to it. The instrument was calibrated with standard solutions and the standard curve was drawn as per standard solution concentration. The aliquot of digested leaf sample (5 ml) was taken in volumetric flask (25 ml capacity) and the volume was made up of 50 ml by adding distilled water. Finally, the sample aliquot was read with flame photometer using the K filter and potassium concentration was worked out with the help of standard curves.

2.5.4 Chlorophyll content

Chlorophyll content in leaves was extracted using 1% dimethylsulfoxide (DMSO) solvent by direct immersion of leaf discs in the solvent. The leaf discs (1 g) were placed in test tube containing 10 ml of solvent. The absorbance of the pigment extract solution was measured at 665 nm and 648 nm using spectrophotometer (Spectrophotometer, HITACHI; Model: U-2000). Thus, chlorophyll concentrations estimated in the

samples were expressed as mg g^{-1} fresh weight [17].

2.6 Data Analysis

The data recorded on leaf nutrient and chlorophyll parameters were statistically analyzed at 5% significance level according to Panse and Sukhatme's standard method [18].

3. RESULTS AND DISCUSSION

3.1 Leaf Nitrogen

The nitrogen content in leaves was significantly affected by weed control methods (Table 1). Paddy straw mulch had the greatest leaf nitrogen concentration (1.88%), followed by black polyethylene mulch (1.85%); nevertheless, no significant difference was identified between these two treatments. Pendimethalin + manual weeding resulted in a considerably greater leaf nitrogen content (1.77 percent) in leaves than pendimethalin @ 1 kg a.i. ha^{-1} . Leaf nitrogen content was 1.71 % in manual weeding and 1.48 % in pendimethalin @ 1 kg a.i. ha^{-1} , respectively. Under weedy control, the lowest leaf nitrogen level was reported (1.42 %).

3.2 Leaf Phosphorous

The phosphorus content of leaves was significantly influenced by weed control methods (Table 1). Paddy straw mulch had the greatest leaf phosphorus concentration (0.30 %); it was comparable to paddy straw mulch and pendimethalin + hand weeding. The leaf phosphorus concentration was 0.29 % in both treatments (black polyethylene mulch and pendimethalin + hand weeding). In comparison to pendimethalin @ 1 kg a.i. ha^{-1} , which had 0.25 % leaf phosphorus, manual weeding registered considerably greater leaf phosphorus concentration in leaves (0.19 %) while weedy check had the lowest leaf phosphorus level.

3.3 Leaf Potassium

Weed control treatments had a substantial impact on leaf potassium level (Table 1). Paddy straw mulch had the highest potassium concentration in leaf (1.15 %), which was statistically comparable to black polyethylene mulch (1.13 %). Manual weeding was at par with pendimethalin @ 1 kg a.i. ha^{-1} with 0.98 % and 0.90% leaf potassium, respectively while

pendimethalin + manual weeding resulted in considerably greater leaf potassium (1.06 %).

Leaf nutrient content in plants are affected by several factors directly or indirectly. Although, plant nutrition has direct and major role in plant nutrient status; the higher temperatures, moisture and organic carbon content due to mulching might have increased biological activities, resulting in faster mineralization and nitrogen availability, as well as high nitrogen translocation from soil to leaves, resulting in increased leaf nutrient levels in mulching treatments [19-20]. Singh and Bal [21] showed that paddy straw mulch had the greatest leaf phosphorus and potassium levels in jujube. Bakshi et al. [22] also found that paddy straw mulch treatment resulted in greater levels of leaf nitrogen, phosphorus, potassium, and calcium in apple. Das et al. [23] found similar findings in litchi as well.

3.4 Chlorophyll Content

Data on leaf chlorophyll content as a function of weed control treatments (Fig. 1) shows that different weed control treatments had substantial effects on chlorophyll content. Paddy straw mulch had the highest chlorophyll concentration in leaves (1.84 mg g⁻¹), although it was statistically comparable to the black polyethylene mulch treatment. The content of chlorophyll in leaves recorded with black polyethylene mulch was 1.81 mg g⁻¹. Pendimethalin + manual weeding and manual weeding recorded 1.76 mg g⁻¹ and 1.70 mg g⁻¹ chlorophyll in leaves, respectively, which was substantially greater than pendimethalin @ 1 kg a.i. ha⁻¹ and manual weeding. Under pendimethalin @ 1 kg a.i. ha⁻¹ and manual weeding, chlorophyll levels in leaves were 1.45 and 1.70 mg g⁻¹, respectively.

Table 1. Effect of weed management practices on leaf Nitrogen, phosphorus and potassium content of grafted apple nursery plants cv. Silver Spur on M9-T337 rootstock

Treatment	Nitrogen (%)	Phosphorus (%)	Potassium (%)
T ₁ : Manual weeding	1.71	0.25	0.98
T ₂ : Pendimethalin @ 1 kg a.i. ha ⁻¹	1.48	0.19	0.90
T ₃ : Pendimethalin @ 1 kg a.i. ha ⁻¹ + manual weeding	1.77	0.29	1.06
T ₄ : Paddy straw mulch (6 cm thick)	1.88	0.30	1.15
T ₅ : Black polyethylene mulch (200 micron)	1.85	0.29	1.13
T ₆ : Weed free	1.83	0.28	1.12
T ₇ : Weedy check	1.42	0.16	0.83
SEm±	0.01	0.01	0.01
C.D(P≤0.05)	0.03	0.02	0.03

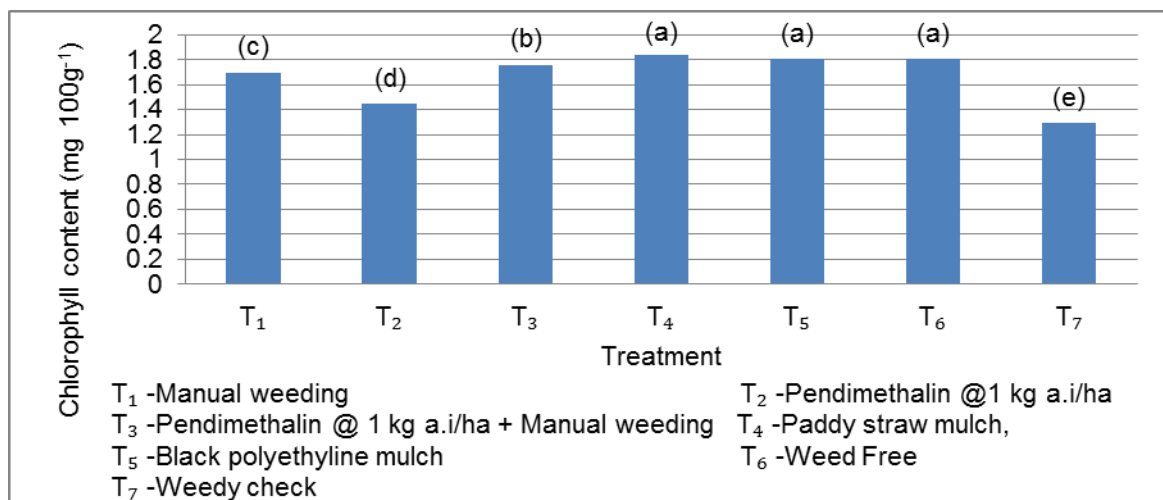


Fig. 1. Effect of weed management practices on Chlorophyll content in the leaves of grafted apple nursery plants cv. Silver Spur on M9-T337 rootstock

The rise in chlorophyll content under mulched treatments might be attributed to an increase in soil microbial population and nitrogen absorption [24]. The results are similar to those of Bons et al. [25] reported that paddy straw mulch produced the maximum leaf chlorophyll concentration in Kinnow mandarin. Improved leaf chlorophyll concentration by mulch application were also reported in strawberry by Deb et al. [26] and Pandey et al. [27] and in raspberry by Krol-Dyrek et al. [28].

4. CONCLUSION

The effect of various weed control approaches on leaf nutrient (nitrogen, phosphorus and potassium) and chlorophyll were found to be significant. Paddy straw mulch resulted in the greatest amount of nutrient (nitrogen, phosphorus and potassium) and chlorophyll content in the leaves of grafted apple nursery plants, although it was on par with black plastic mulch. It is concluded that weed control in apple nurseries using paddy straw mulch and black polyethylene mulch was found to be helpful in terms of improved leaf nutrient (nitrogen, phosphorus, and potassium) and chlorophyll content in the plants.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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