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RESEARCH ARTICLE

BIO-CHEMICAL AND MINERAL CONSTITUENTS OF MULBERRY LEAF RAISED THROUGH ORGANIC BASED NUTRIENTS IN RED LOAMY SOIL

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Abstract

The results of the study revealed that, biochemical and mineral constituents of mulberry were significantly better with the plots supplied with vermicompost (Equivalent to 50 % of Rec. FYM) + FYM (50% of Rec. FYM) + N-biofertilizer + P-biofertilizer + 200N + 110P + 140 K kg/ha/yr, followed by sheep manure (Equivalent to 50% of Rec. FYM) + FYM (50 % of Rec. FYM) + N-biofertilizer + P-biofertilizer + 200N + 110P + 140 K kg/ha/yr and the values were lower in control.

Introduction

Mulberry (*Morus* spp.) is a perennial and high biomass producing plant, continues to grow throughout the year in tropics. The continuous production of mulberry for a long time results in gradual reduction in leaf yield and quality (Rashmi *et al*., 2009). The silkworm, *Bombyx mori* L. being monophagous insect, derives almost all the nutrients for growth and development from the mulberry leaf. It has been estimated that, nearly 70%of the silk proteins are derived from mulberry leaves. Hence, silkworms should be fed with good quality mulberry leaves in abundant quantity for the successful cocoon production (Vijaya *et al*., 2009).

The leaf yield and quality of mulberry depends on the soil type, plant variety, and availability of plant nutrients and agro-ecological conditions, which reflects on the quality of silk production. In India, mulberry is largely cultivated for leaf production and contributes to an extent of 38.20 per cent for successful cocoon crop production (Miyashita, 1986). Application of organic manures to the soil either in the form of organic or inorganic inputs are to supply nutrients to the plants. Organic manures such as FYM, compost, green manures, oil cakes and biofertilizers, etc., contain comparatively smaller quantities of plant nutrients apart from being bulky in nature (Shivaprakash and Narayanagowda, 2006). Mulberry leaf productivity is highly dependent on plant nutrients like NPK and is known to respond well to the addition of organic manures. The chemical fertilizers are becoming costlier day by day due to escalating costs and scarce availability of commodities. Further, intensive mulberry cropping system causes depletion of nutrients in soil and excess usages of inorganic fertilizers and pesticides caused deleterious effect on soil health (Shashidhar *et al*., 2009).

Organic sources release the nutrients slowly and limit the loss of plant nutrients by leaching and volatilization under adverse soil conditions (alkaline or acidic). Manures are plant and animal wastes that are used as sources of plant nutrients and release nutrients after their decomposition. Continuous use of chemical fertilizers affects soil health in addition to environmental pollution, impair the balanced availability of different plant nutrients which results in wide spread deficiencies including those of micronutrients (Krishna and Bongale, 2001). Hence, it is imperative to integrate various sources of plant nutrients through application of manures, chemical and bio-fertilizers in optimum quantity to enhance the qualitative and quantitative characters of mulberry.

MATERIALS AND METHODS

Study area

A field experiment was conducted in farmer's field of Molakalmur taluk, Chitradurga district, Karnataka, during monsoon season in order to estimate the bio-chemical and mineral constituents in mulberry raised through organic based nutrients (FYM, compost, vermicompost, sheep manure and biofertilizer) in established irrigated mulberry (V_1) with a spacing of (150 + 90 cm) x 60 cm. The soil of the experimental site was red loamy in texture and the plot is situated in the Central dry zone (Zone-IV) of Karnataka and lies between 13^0 34' to 15^0 02' North latitude and 75^0 37' to 77^0 01'East longitude and having temperature of 37^0C (Max.) and 15^0C (Min.). The experiment was laid out in Randomized Block Design consisting of eight treatments with three replications. Treatments were imposed to irrigated mulberry garden based on the recommended FYM @ 20 MT/ha/yr. However, compost, vermicompost and sheep manures were applied based on the recommended quantity of FYM (MT/ha/yr). The N biofertilizer (*Azospirillum brasilense*) (@ 23 kg/ha/yr) and P biofertilizer (*Aspergillus awamori*) (@ 5 kg/ha/yr) and chemical fertilizers (NPK) were applied in kg/ha/yr. The cultivation practices were followed as per the recommended package developed for mulberry cultivation under irrigated condition (Dandin and Giridhar, 2010).

Treatments

T_0 Recommended FYM (20MT/ha/yr)+350 N+140 P+140 K kg/ha/yr (Control)

T_1 Rec. FYM+N-biofertilizer+P-biofertilizer+200 N+110 P+140 K

T_2 Compost (Equivalent to 50 % of Rec. FYM+FYM (50% of Rec. FYM) + 350N+140P+140K

T_3 Vermicompost (Equivalent to 50 % of Rec. FYM) + FYM (50 % of Rec. FYM) + 350 N+140 P+140 K

T_4 Sheep manure (Equivalent to 50 % of Rec. FYM) + FYM (50 % of Rec. FYM) +350 N +140 P+140 K

T_5 Compost (Equivalent to 50 % of Rec. FYM)+FYM(50% of Rec. FYM)+N-biofertilizer+P-biofertilizer +200 N+110 P+140 K

T_6 Vermicompost (Equivalent to 50 % of Rec. FYM) +FYM (50 % of Rec. FYM) +N-biofertilizer+P-biofertilizer+200 N+110 P+140 K

T_7 Sheep manure (Equivalent to 50% of Rec. FYM) +FYM (50 % of Rec. FYM)+N-biofertilizer+P-biofertilizer+200 N+110 P+140 K.

Collection of mulberry leaf samples: Mulberry leaf samples at different heights of the plant (top, middle and bottom) were collected in paper bags at 60 days after pruning and composite leaf samples were made. Leaves were shade dried for three days and then dried in hot air oven at 70^0C for one hour and were ground into powder for chemo-assay. The leaves obtained from different treatments were used for estimation of bio-chemical and mineral constituents following standard procedures:

Leaf moisture (%): Moisture content of the leaf was estimated through gravimetric method by taking the difference between fresh and dry weight (A.O.A.C., 1980) and was expressed on fresh weight basis.

Chlorophyll (mg/g): Chlorophyll content of the mulberry leaf was estimated following the procedure outlined by Hiscox and Israelstam (1979) using spectrophotometer. Chlorophyll 'a', 'b' and total chlorophyll were computed using the standard formulae (Arnon, 1949) and was expressed on fresh weight basis.

Protein (mg/g): Protein content of the leaf was estimated following the procedure of Lowry *et al.* (1951) and was expressed on oven dry weight basis.

Total sugars (mg/g): The total sugar content of the leaf was estimated following the method of Dubios *et al.* (1956) and was expressed on oven dry weight basis.

Nitrogen (%): The nitrogen content was estimated employing Micro-Kjeldhal method as outlined by A.O.A.C. (1980) and was expressed on oven dry weight basis.

Phosphorus (%): The phosphorus content was determined using spectrophotometer and as per the procedure outlined by the method of Jackson (1973) and was expressed on oven dry weight basis.

Potassium (%): The potassium content in the digested extract was determined using flame photometer and as per the method of Jackson (1973) and was expressed on oven dry weight basis.

Calcium and magnesium (%): The calcium and magnesium contents were determined by the EDTA or Versenate-titration method as described by Jackson (1973).

Sulphur (%): The sulphur content in the di-acid digested sample was estimated by turbidometric method as outlined by Jackson (1973).

RESULTS AND DISCUSSION

The results on the estimation of biochemical and mineral constituents in mulberry cultivated through organic based nutrients in red loamy soil are discussed hereunder.

Bio-chemical constituents of mulberry leaf

Leaf moisture (%): Leaf moisture has a greater role in determining the palatability of leaf during silkworm rearing, as it varies greatly with the cultivation practices and maturity of leaf. In the current investigation, mulberry raised by applying different sources of organic manures, chemical and bio-fertilizers at recommended quantity with different proportion did not exert significant variations with respect to leaf moisture. However, leaf moisture was comparatively better with the application of vermicompost (Equivalent to 50 % of recommended FYM)+FYM (50 % of Rec. FYM)+N-biofertilizer+P-biofertilizer+200N+110P+140K to red loamy soil and it was less in control [Rec. FYM (20 MT/ha/yr) +350N+140P+140K kg/ha/yr] at 60^{th} days after pruning (Fig. 1). The enhancement of leaf moisture due to application of organic manures might be due to enhancement of organic matter and water holding capacity in the soil, thereby, absorption of water by plant increases (Rao *et al*., 2011).The current observations are similar to the findings of Babu *et al*. (2013), who found that the leaf quality with organic treatment was on par with that of conventional farming and the mulberry leaf produced through organic cultivation has no negative impact on leaf moisture.

Chlorophyll (mg/g): The chlorophyll content in leaf is greatly determined by the rate of photosynthesis in the plant. Chlorophyll in the presence of light produces red fluorescent protein which induces resistance in silkworm from viral diseases. Total chlorophyll content was significantly high with the application of vermicompost (Equivalent to 50 % of recommended FYM)+FYM (50 % of Rec. FYM)+N-biofertilizer+P-biofertilizer+200N+110P+140Kin red loamy soil followed by application of sheep manure (Equivalent to 50% of Rec. FYM)+FYM (50 % of Rec. FYM)+N-biofertilizer+P-biofertilizer+200N+110P+140Kat 60^{th} days after pruning (Fig. 2).However, mulberry supplied with varied sources of organic manures, chemical and bio-fertilizers did not exhibit variations with respect to chlorophyll ‘a’ and chlorophyll ‘b’ contents. The improvement in chlorophyll content indicates the photosynthetic efficiency of T3 plants. Singhal *et al*. (2000) opined that nitrogen plays a greater role in improving the chlorophyll synthesis, as it is an essential constituent of photosynthesis. Similarly, Rashmi *et al.* (2009) observed that, the total chlorophyll content was maximum with the application of chemical fertilizers along with biofertilizers, compost, vermicompost, green manure and castor cake. Further, Vijaya *et al.* (2009) also reported that the increased chlorophyll content in mulberry leaf was in recommended dose of fertilizers and foliar nutrition. The observations are in close conformity with the findings of Rao et al. (2011), wherein application of chemical fertilizer along with FYM, green manures and seri-compost to mulberry increased the total chlorophyll content.

Protein and total sugars (mg/g): Protein and sugars in mulberry leaf determines the feeding value of silkworm, as the silk production is greatly influenced by these two bio-chemical constituents. In the current investigation, protein content in mulberry leaf did not differ statistically in response to application of varied sources and levels of organic manures, chemical and bio-fertilizers. In contrast, total sugar content was statistically more in the mulberry plot that received vermicompost (Equivalent to 50 % of Rec. FYM)+FYM (50 % of Rec. FYM) + N-biofertilizer + P-biofertilizer + 200N + 110P + 140K,followed by application of sheep manure (Equivalent to 50% of Rec. FYM) + FYM (50 % of Rec. FYM)+N-biofertilizer+P-biofertilizer+200N+110P+140K and it was low in control[Rec. FYM (20 MT/ha/yr) +350N+140P+140K kg/ha/yr]at 60^{th}days after pruning (Fig. 3).The enhanced total sugar content in mulberry leaf was attributed to the improved mineralization resulting in enhanced production of plant growth substances and enzyme activity in mulberry. These results are in close conformity with the work of Vijaya *et al.* (2009), who reported that the application of NPK to soil and foliar spray increased the total sugars in mulberry leaves. Similarly, Rashmi *et al*. (2009) recorded higher total sugar content in mulberry due to the application of chemical fertilizers along with biofertilizers, compost, vermicompost, green manures and castor cake.

Major nutrients in mulberry leaf

Nitrogen (%): Statistical variation was observed among the different sources of organic manures when applied to mulberry in respect of nitrogen content in mulberry leaf. Nitrogen content was significantly higher in the mulberry leaves raised with vermicompost (Equivalent to 50 % of Rec. FYM)+FYM (50 % of Rec. FYM) + N-biofertilizer + P-biofertilizer + 200N + 110P + 140K, followed by application of sheep manure (Equivalent to 50% of Rec. FYM) + FYM (50 % of Rec. FYM)+N-biofertilizer+P-biofertilizer+200N+110P+140K and it was lower with control[Rec. FYM (20 MT/ha/yr) +350N+140P+140K kg/ha/yr] at 60^{th} days after pruning (Fig. 4).

Phosphorus (%): Significantly higher phosphorus content in leaf was noticed with mulberry raised by applying vermicompost (Equivalent to 50 % of Rec. FYM)+FYM (50 % of Rec. FYM)+N-biofertilizer+P-biofertilizer+200N+110P+140K kg/ha/yr, followed by application of sheep manure (Equivalent to 50% of Rec. FYM)+FYM (50 % of Rec. FYM)+N-biofertilizer+P-biofertilizer+200N+110P+140K at 60 days of pruning mulberry. However, lower phosphorus content was recorded in control [Rec. FYM (20 MT/ha/yr) +350N+140P+140K kg/ha/yr] (Fig. 4).

Potassium (%): Potassium content in mulberry leaf was statistically more with mulberry raised by applying vermicompost (Equivalent to 50 % of Rec. FYM)+FYM (50 % of Rec. FYM)+N-biofertilizer+P-biofertilizer+200N+110P+140K kg/ha/yr, followed by application of sheep manure (Equivalent to 50% of Rec. FYM)+FYM (50 % of Rec. FYM)+N-biofertilizer+P-biofertilizer+200N+110P+140K and it was less in control [Rec. FYM (20 MT/ha/yr) +350N+140P+140K kg/ha/yr] (Fig. 4).

Increased nitrogen, phosphorus and potassium contents in leaves might be due to increased availability of nutrients in soil due to application of organic manures, chemical and bio-fertilizers. This is an indication for uptake of plant nutrients due to the synergetic effect of various beneficial microorganisms flourishing in the soil due to addition of organic manures. Rajanna *et al*. (2000) reported that, the application of recommended FYM+NPK recorded significantly higher macro nutrients in mulberry leaf followed by sheep manure + recommended FYM. Similarly, Singhvi *et al*. (2000) and Rajegowda *et al*. (2000) reported that the application of seri-boost to mulberry increased the phosphorus content in leaf. Vijaya *et al*. (2009) recorded increased macro nutrients in mulberry leaves with the application of 100% recommended dose of fertilizers + foliar nutrition (@ 2%). Rashmi *et al*. (2009), who observed that the application of chemical fertilizers along with biofertilizers, compost, vermicompost, green manures and castor cake, increased the macro nutrients in leaves.

Secondary nutrients in mulberry leaf

Calcium (%): Calcium content in mulberry leaf varied significantly among the different sources of organic manures when applied to mulberry. Calcium content was significantly more in the mulberry leaf raised with the application of vermicompost (Equivalent to 50 % of Rec. FYM)+FYM (50 % of Rec. FYM) + N-biofertilizer + P-biofertilizer + 200N + 110P + 140K, followed by sheep manure (Equivalent to 50% of Rec. FYM) + FYM (50 % of Rec. FYM)+N-biofertilizer+P-biofertilizer+200N+110P+140K 60th days after pruning. Notably, calcium content was less in control [Rec. FYM (20 MT/ha/yr) +350N+140P+140K kg/ha/yr] (Fig. 5).

Magnesium (%): Significantly higher magnesium content in mulberry leaf was recorded with the application of vermicompost (Equivalent to 50 % of Rec. FYM)+FYM (50 % of Rec. FYM) + N-biofertilizer + P-biofertilizer + 200N + 110P + 140K,followed by application of sheep manure (Equivalent to 50% of Rec. FYM) + FYM (50 % of Rec. FYM)+N-biofertilizer+P-biofertilizer+200N+110P+140Kat 60th days after pruning. However, it was lower in control [Rec. FYM (20 MT/ha/yr) +350N+140P+140K kg/ha/yr] (Fig. 5).

Sulphur (%): Significantly increased sulphur content in mulberry leaf was observed with the application of vermicompost (Equivalent to 50 % of Rec. FYM)+FYM (50 % of Rec. FYM) + N-biofertilizer + P-biofertilizer + 200N + 110P + 140K, followed by application of sheep manure (Equivalent to 50% of Rec. FYM) + FYM (50 % of Rec. FYM)+N-biofertilizer+P-biofertilizer+200N+110P+140Kat 60th days after pruning. However, it was less in control [Rec. FYM (20 MT/ha/yr) +350N+140P+140K kg/ha/yr] (Fig. 5).

Application of recommended FYM+NPK recorded significantly more secondary nutrients in mulberry leaf followed by sheep manure + recommended FYM (Rajanna *et al*., 2000). Sunil (2005) reported that the combination of organic manures, bio-inoculants and inorganic fertilizers recorded significantly higher nutrients in leaves of S36 and M5 mulberry varieties. Naika (2008) reported that, the secondary nutrients in V1 mulberry was higher with enriched vermicompost + recommended P and K. Increased secondary nutrients in mulberry leaf was found with combination of organic manures, biofertilizers, green manures and chemical fertilizers (Shashidhar, 2009). Further, Rashmi *et al*. (2009) reported that, the application of chemical fertilizers along with biofertilizers, compost, vermicompost, green manures and castor cake, increased the calcium, magnesium and sulphur contents in mulberry leaves.

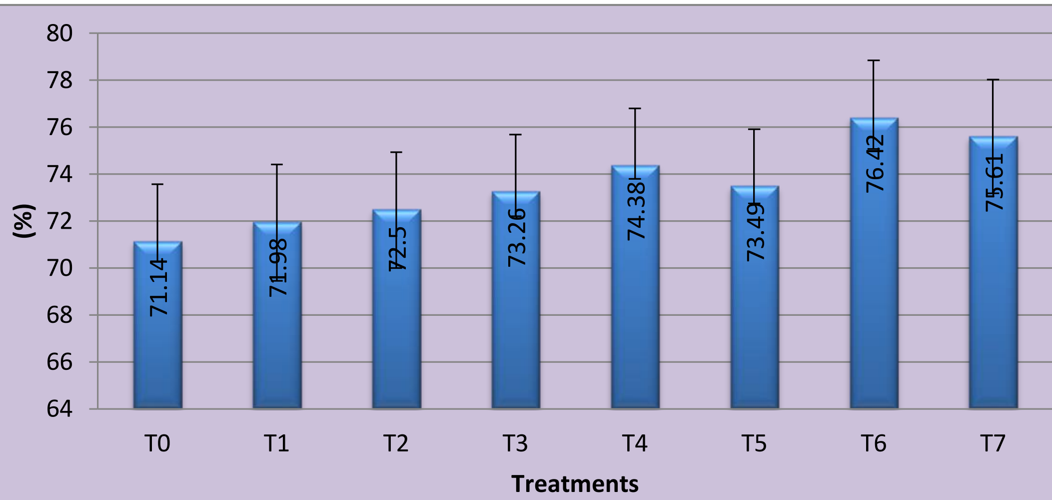

Fig. 1: Leaf moisture in mulberry as influenced by organic based nutrients in red loamy soil

Statistical tests	Statistical values (ANOVA)
F-test	--
S.E(m) ±	1.698
S.E(d) ±	2.402
C.D. 5%	Non-significant
C.V. (%)	3.997

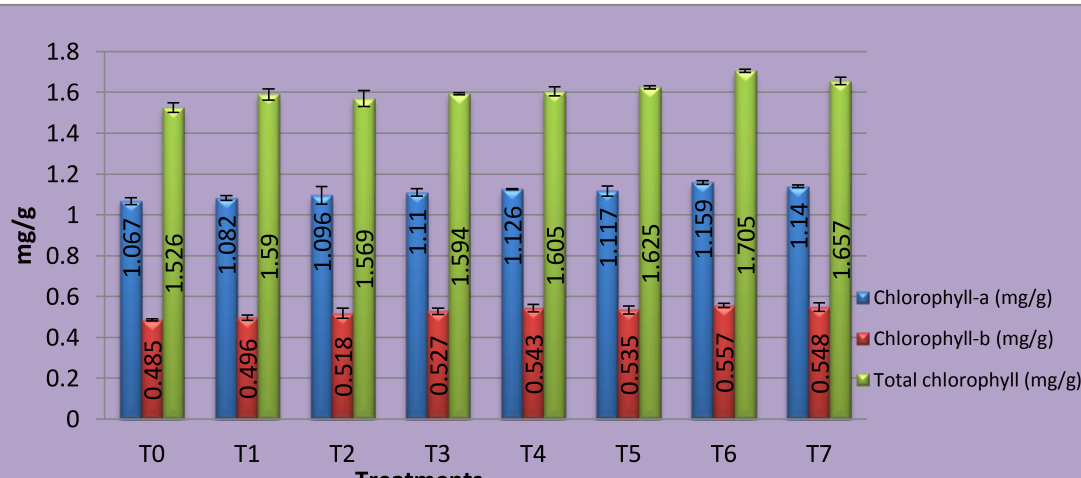

Fig. 2: Chlorophyll content in mulberry as influenced by organic based nutrients in red loamy soil

Statistical tests	Statistical values (ANOVA)		
F-test	--	--	* (P≤0.05)
S.E(m) ±	0.021	0.017	0.022
S.E(d) ±	0.029	0.025	0.031
C.D. 5%	Non-significant	Non-significant	0.066
C.V. (%)	3.194	5.713	2.363

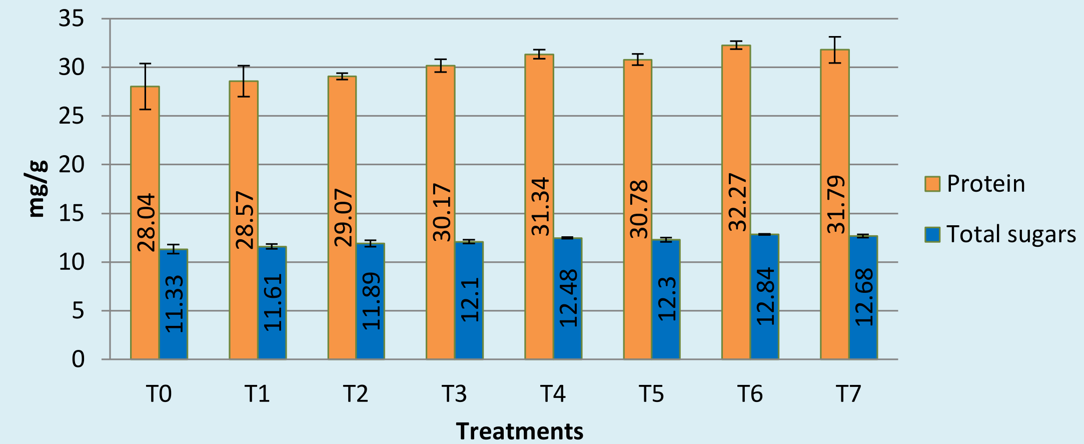

Fig.3: Protein and total sugars in mulberry leaf as influenced by organic based nutrients in red loamy soil

Statistical tests	Statistical values (ANOVA)	
F-test	--	* (P≤0.05)
S.E(m) ±	1.182	0.249
S.E(d) ±	1.672	0.352
C.D. 5%	Non-significant	0.753
C.V. (%)	6.768	3.551

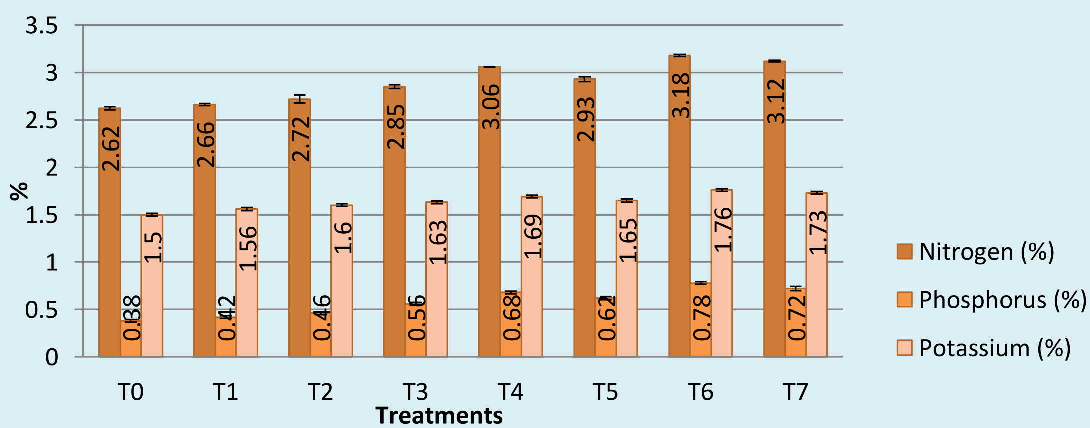

Fig. 4: Major nutrient status in mulberry leaf as influenced by organic based nutrients in red loamy soil

Statistical tests	Statistical values (ANOVA)		
F-test	* (P≤0.05)	* (P≤0.05)	* (P≤0.05)
S.E(m) ±	0.016	0.016	0.015
S.E(d) ±	0.022	0.023	0.022
C.D. 5%	0.047	0.049	0.046
C.V. (%)	0.939	4.821	1.613

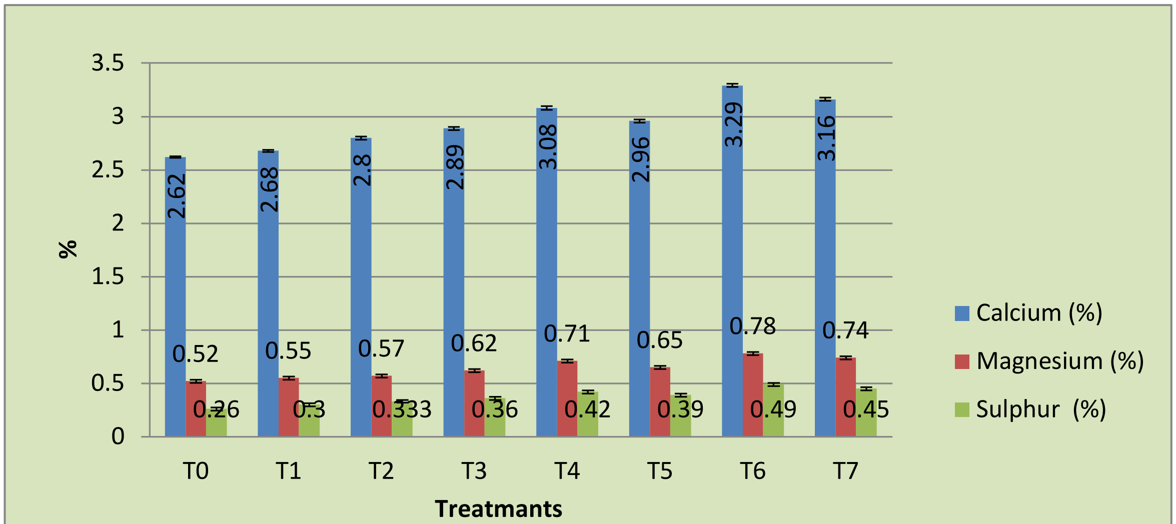

Fig. 5: Secondary nutrient status in mulberry leaf as influenced by organic based nutrients in red loamy soil

Statistical tests	Statistical values (ANOVA)		
F-test	* (P≤0.05)	* (P≤0.05)	* (P≤0.05)
S.E(m) ±	0.014	0.015	0.015
S.E(d) ±	0.020	0.022	0.021
C.D. 5%	0.042	0.046	0.045
C.V. (%)	0.817	4.118	6.878

CONCLUSION

The current investigation inferred that, application of organic manures through vermicompost and FYM(followed by sheep manure and FYM; compost and FYM) in equal proportion along with recommended doses of chemical and bio-fertilizers to mulberry garden produced better quality of leaves i.e., by way of enhancement of bio-chemical and mineral nutrients. Thus, in the event of shortage /non-availability of FYM, 50% recommended dose of FYM can be compensated through use of vermicompost / sheep manure / compost to suffice the manurial requirement of mulberry.

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