Influence of inorganic, organic and bio fertilizer on chlorophyll content, relative water and EC content of leaf of host plant of Tasar silkworm (*Antheraea mylitta* Drury)

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Abstract
The research was done to find out the influence of Inorganic, Organic and Biofertilizer on Chlorophyll content, Relative water and EC content of leaf of host plant of Tasar Silkworm (*Antheraea mylitta* Drury). The results showed that application of different combination of manure significantly influenced the Chlorophyll content, Relative water and EC content. The RWC was found highest in T_{17}(85.2%) was applied with 100%RDF through fertilizer +2kg vermicompost+ PSB followed by T_{18}(85.02%) and T_3(84.99%). The EC was found highest in T_{19}(0.09405 dSm^{-1}) followed by T_{18}(0.0922 dSm^{-1}). After 10 min the EC was found to be highest in T_{19}(0.245dSm^{-1}) followed by T_6(0.230dSm^{-1}). The Chl 'a' was recorded high in T_{16}(4.70mg/g), Chl 'b' in T_7 (3.18 mg/g) and total chlorophyll was recorded highest in T_7(7.68 mg/g) followed by T_{16}(7.55 mg/g). The obtained results showed that combined application of manure have significant affect on chlorophyll content, Relative water and EC content of leaf.

Keywords: biofertilizer, chlorophyll, relative water, silkworm and vermicompost

Introduction

Silk farming is a commercial cultivation providing valuable foreign exchange (www.imotforum.com) and employment and livelihood to approximately 9.4 million persons in rural and semi-urban areas. It is the oldest industry in the country. All the five known commercial silks namely Mulberry, Tropical Tasar, Oak-Tasar, Eri and Muga are produced by India making it unique in the world (Central Silk board).Good quality of Silk production depend on plant nutrition. For optimal growth of plant, nutrients must be present in sufficient and balanced quantities. Fertilization of the soil are done to maintain the natural fertility and which also act as a sources of plant nutrient (Purwanto and Junaidah, 2015) [26]. Use of organic fertilizer can meet the nutrient requirements for sustainable production. Management of organic and biofertilizer reduces the potential damage than mineral fertilizer (Ahmed *et al*., 2011) [1]. Biofertilizer application in agriculture is a sustainable way of increasing crop yields and reduces production cost (Wali Asal, 2010) [37]. Bio-fertilization is a safe process for human, animal and environments which lower pollution and fertilization cost. It helps in improving the soil biota and also reduces the sole use of chemical fertilizers (Sabashini *et al*., 2007) [29]. Organic material act as soil conditioners, they not only serve as source of plant nutrients but also improve soil physical properties, as evidenced by increased water infiltration, water holding capacity, aeration and permeability, soil aggregation and rooting depth and by decreasing soil crusting, bulk density and erosion (Allision 1973, USDA 1978). Application of organic and inorganic fertilizers to the soil helps in growth of plants by supplying nutrients and thus affects the physiological processes of plants (Amujoyegbe *et al*, 2007) [4].

RWC indicate the plant water status and was used instead of plant water potential. It indicate the balance between absorbed water by plant and consumed through transpiration (M. Hassanzadeh *et al*., 2009) [20]. Plant having high RWC, are more resistant against drought stress (Schonfeld *et al*., 1988) [30]. Photosynthetic capacity decreases usually at relative water content below 70% which in many plants correspond to severe wilting of leaves but these inhibitory effects are still reversible down to 30%-70% of relative water content (Kaiser, 1987) [15].

EC is related to amount of ions available in the root zone of plant (Nemail and Van Iersel, 2004) [24]. The salt concentration and electrolyte concentration index of the solution are determined by EC (Kozai *et al*., 2015; Lu *et al*., 2017) [16, 19]. The EC depend on the environmental conditions and for different crop it has different optimal EC (Le Bot *et al*., 1998; Sonneveld and Voogt, 2009) [17, 34]. Higher EC causes environmental pollution by increasing the discharge of nutrients into the environment and also hinder nutrient uptake by increasing the osmotic pressure. Plant health and yield are severely affected by lower EC (Signore *et al*., 2016; Lu *et al*., 2017) [32, 19].

Chlorophylls in the plant are the most important green pigments for photosynthetic process (Bhatia and parashar, 1997) [7]. Five types of chlorophyll occur in plants a, b, c, d and e out of these, in higher plant two chlorophyll i.e. and b are found. chlorophyll a is termed as universal photosynthetic pigment and also known as primary photosynthetic pigment because it convert light into chemical or electrical energy which is the primary reaction centre of photosynthesis. Other pigments are accessory pigments. They absorb light energy of different wavelengths and pass the energy to chlorophyll a through electron spin resonance (Bhatia and Tyagi, 2006) [6].

Leaf chlorophyll content is indicator of photosynthetic capability of plant tissue (Nageswara *et al*. 2001; Write *et al*., 1994) [23] and also provide indirect but accurate estimate of nutritional status of plant (Vijay paul *et al*., 2017) [36]. Chlorophyll green coloration is related to the amount of nutrient absorbed by the plant from soil (Roy and Singh, 2006) and also observed that fertilization with organic manure increase chlorophyll content than inorganic fertilizer. Photosynthetic activity and crop yield increase with increased chlorophyll content in leaf (Siavoshi and Laware, 2013) [31]. This research was done to find the influence of inorganic, organic and biofertilizer on chlorophyll content, Relative water and EC content of leaf of host plant of Tasar Silkworm (*Antheraea mylitta* Drury).

Materials and Methods

The present study was carried out in the field of Central Tasar Research & Training institute, Nagri, Ranchi to determine the influence of inorganic, organic and biofertilizer on Chlorophyll content, Relative water and EC content of leaf of host plant of Tasar Silkworm (*Antheraea mylitta* Drury). Twenty different combination with three replication were laid out in Randomized Complete Block Design (Table1).

Table1: Treatment Details

Treatments	Treatments detail
T_1	Absolute Control
T_2	Control with recommended dose fertilizer(RDF)
T_3	50% RDF through fertilizer+50% through vermicompost
T_4	75% RDF through fertilizer+25% through vermicompost
T_5	100% RDF through fertilizer+2% through vermicompost
T_6	50%RDF+Azotobacter
T_7	75%RDF+Azotobacter
T_8	100%RDF+Azotobacter
T_9	50% RDF+ Phosphorus solubilizing bacteria(PSB)
T_{10}	75%RDF+PSB
T_{11}	100%RDF+PSB
T_{12}	T_3+Azotobacter
T_{13}	T_4+Azotobacter
T_{14}	T_5+Azotobacter
T_{15}	T_3+PSB
T_{16}	T_4+PSB
T_{17}	T_5+PSB
T_{18}	T_3+Azotobacter+PSB
T_{19}	T_4+Azotobacter+PSB
T_{20}	T_5+Azotobacter+PSB

Sample Collection

Fresh leaf sample from each treatment were collected and bought to the laboratory for analysis of different parameters.

Biochemical Parameters

Estimation of Chlorophyll content

Chlorophyll content of the leaves was determined by procedure described by Hiscox and Israelstam, 1979. The chlorophyll a and b content of leaf was computed using formula suggested by Arnon (1949) [5].

$$\text{Chlorophyll 'a' (mg/g)} = \frac{12.7(A663)-2.69(A645)}{1000*W} * V$$

$$\text{Chlorophyll 'b' (mg/g)} = \frac{22.9(A645)-4.68(A663)}{1000*W} * V$$

$$\text{Total Chlorophyll (mg/g)} = \frac{20.2(A645)+8.02(A663)}{1000*W} * V$$

Physiological Parameters

Relative water content

The water potential of the leaf tissue was estimated according to the method of Slatyer (1955) [33]. About 1 gm of fresh leaves sample was weighed and dipped in 20 ml distilled water for three hour. After three hours turgid weight of the leaves was taken and then kept for drying in 60°C in Hot air oven till the weight become constant. The RWC was computed as

$$\text{RWC\%} = \frac{\text{Fresh weight of leaf-Dry weight of leaf}}{\text{Turgid weight-Dry weight}} * 100$$

Electrical Conductivity of leaf

The electrical conductivity of the leaf was estimated to the method of Bower and Wilcox, 1965. About 1 gm of fresh leaf was taken than dipped in 50ml distilled water for 10min. After 10 min EC was measured using electrical conductivity meter and then kept in water bath at 60ºC for 10 min than again EC was measured.

Statistical Analysis

The data were analysed using Descriptive Statistics and SPSS20. Significant difference among mean were distinguished according to the Duncan's Multiple Test Range (Duncan, 1955) [10].

Result and Discussions

Physiological Character

a. Relative Water Content

The relative water content was seen to be significantly affected by different treatment. The Relative Water Content ranged from 72.646-87.73% with highest(85.2%) in T_{17} was applied with 100% RDF through fertilizer+ 2kg Vermicompost+ Phosphate Solubilizing Bacteria(PSB) followed by T_{18}(85.02%) and T_3(84.99%). It was found that the RWC affect the chlorophyll pigment. Leaf pigment get damaged by water deficit as reported by (Montag U and Woo, 1990; Nilsen and orcutt, 1996) [25], due to production of Reactive Oxygen Species (ROS) such as O_2^- and H_2O_2 lead to lipid peroxidation and thus chlorophyll destruction (Mirnoff, 1993; Foyer *et al*., 1994) [21, 12]. The chlorophyll is destroyed by water deficit and formation also prevented (Lessani and Mojtahedi, 2002) [18].

Table 2: Showing the effect of different treatment on RWC

Treatments	Relative water content (%)	
	Mean	Rank
T1	75.61^h	20
T2	76.95^h	19
T3	84.99^c	3
T4	77.77^h	18
T5	84.08^c	5
T6	80.48^{ef}	11
T7	78.33^g	15
T8	79.75^f	12
T9	83.83^d	7
T10	78.22^{gh}	17
T11	81.85^d	8
T12	81.16^{de}	10
T13	79.7^f	14
T14	83.88^c	6
T15	79.7^f	13
T16	81.48^d	9
T17	85.2^a	1
T18	85.02^b	2
T19	78.32^g	16
T20	84.31^c	4
Mean	81.0315	
S.Em±	0.4306	
Range	72.646-87.731	
CD (95.0%)	0.8617	

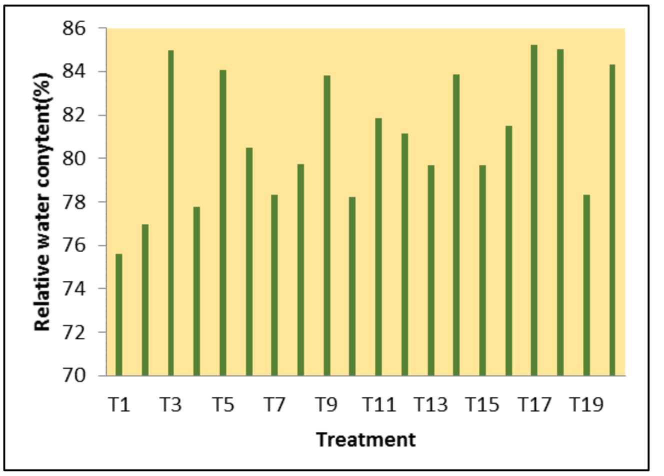

Fig 1: Influence of different treatment on RWC

b. Electrical Conductivity of leaf

The EC content of the leaf also found to be significantly affected by different nutrient treatment application. The EC content of leaf ranged from 0.03097-0.0979dSm^{-1}. The EC content of T_{19} was recorded highest (0.09405dSm^{-1}) followed by T_{18} (0.0922dSm^{-1}) and T_5 (0.0828dSm^{-1}). After 10 min of water bath treatment the EC of the leaf ranged from 0.05456-0.2527dSm^{-1} with highest recorded in T_{19} (0.245 dSm^{-1}) followed by T_6 (0.230 dSm^{-1}) and T_{18} (0.2115 dSm^{-1}). The leaf chlorophyll content are also get affected by EC content. The chlorophyll content increase gradually as the EC content increase and decrease with lower EC content (Ding X *et al*., 2018) [11].

Table 3: Effect of different treatment on EC content

Treatments	EC	EC after 10min
T_1	**0.0325**	0.05628
T_2	0.03265	0.0557
T_3	0.0629	0.1774
T_4	0.037	0.0673
T_5	0.0828	0.1721
T_6	0.077	0.230
T_7	0.03247	0.08525
T_8	0.07325	0.1932
T_9	0.034	0.06335
T_{10}	0.0374	0.0866
T_{11}	0.04905	0.0672
T_{12}	0.03795	0.07855
T_{13}	0.03635	0.0603
T_{14}	0.042	0.0805
T_{15}	0.0429	0.0837
T_{16}	0.03905	0.07315
T_{17}	0.0391	0.0989
T_{18}	0.0922	0.2115
T_{19}	0.09405	.245
T_{20}	0.0807	.19155
Mean	0.0527	0.1188
S.Em±	0.0027	0.00837
Range	0.03097-0.0979	0.05456-0.2527
CD (95.0%)	0.00559	0.01675

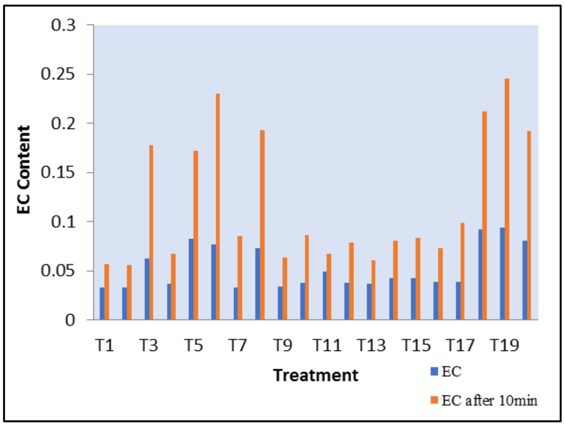

Fig 3: Influence of different treatment on EC content

Biochemical Characters

a. Chlorophyll content

Effect of inorganic, organic and biofertilizer were significant on leaf chlorophyll content. Relative water content and Electrical Conductivity were found to effect significantly the Chlorophyll content. Chlorophyll 'a' content was higher in T_{16} (4.70 mg/g) which was applied with 75%RDF through fertilizer+25% through vermicompost+ Phosphate Solubilizing Bacteria(PSB) over control T_1(1.05 mg/g). Chlorophyll 'b' content recorded high in T_7 (3.18 mg/g) was treated with 75% RDF + *Azotobacter*. Total chlorophyll content of fresh leaf ranged from 1.719-8.049 mg/g with highest value in T_7 (7.68mg/g) followed by T_{16} (7.55mg/g). The increase in chlorophyll content due to fertilization was also reported by (Amany S. Al-Erwy *et al*., 2016) [3]. Fertilization with different manure increases the N content in the soil. Which helps in good N supply to the plant. Nitrogen is a important structural element of chlorophyll and protein molecules thus affecting the formation of chloroplasts and chlorophyll accumulation (Ray Tucker, 2004) [27]. Stability of chlorophyll in plants is affected by nitrogen concentration and is the important mineral in chlorophyll biosynthesis. Excesses of nitrogen decreases chlorophyll content in leaf (Bojovic and Stojanovic, 2005) [8].

Table 4: Chlorophyll content after different treatment

Treatments	Chl 'a' (mg/g)	Chl 'b' (mg/g)	Total Chl (mg/g)
T_1	1.05	0.66	1.72
T_2	2.76	2.38	5.14
T_3	2.69	2.27	4.97
T_4	1.11	0.77	1.99
T_5	2.16	1.92	4.07
T_6	2.03	1.73	3.76
T_7	4.49	3.18	7.68
T_8	2.39	2.12	4.51
T_9	3.09	2.06	5.15
T_{10}	2.65	2.57	5.31
T_{11}	2.29	2.20	4.49
T_{12}	3.46	2.51	5.97
T_{13}	2.96	2.52	5.48
T_{14}	2.78	2.06	4.85
T_{15}	2.13	1.73	3.86
T_{16}	4.70	2.84	7.55
T_{17}	4.24	2.66	6.91
T_{18}	3.74	2.33	6.06
T_{19}	3.88	2.64	6.52
T_{20}	4.66	2.64	7.29
Mean	2.96	2.189	5.164
S.Em±	0.136	0.079	0.2081
Range	1.041-4.761	0.6368-3.313	1.719-8.049
CD (95.0%)	0.273	0.1584	0.4164

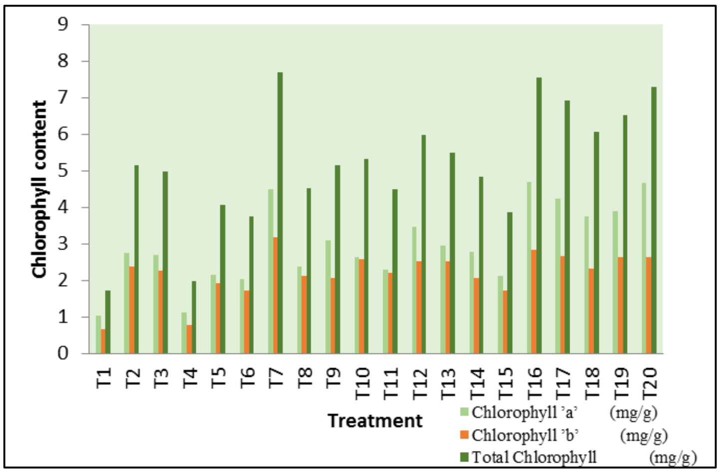

Fig 4: Influence of different treatment on chlorophyll content

Conclusion

From the above data collected it can be concluded that application of inorganic, organic and biofertilizer significantly influenced the leaf chlorophyll content, Relative water and EC content than the untreated plant. Combined application of all three fertilizer are most effective in increasing the nutrient content of leaf. Therefore, combined application of fertilizer may be better than the use of chemical fertilizer alone. Which shows negative effect on soil health and environment.

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