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Influence of potassium humate on *Morus alba* and analysis their nutrients content

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Abstract

We aimed to the influence of potassium humate on growth rate and nutritional value of Mulberry plant is studied. Potassium humate is extracted from lignite by treating with Potassium hydroxide. In the present study, Mulberry plants were grown in different concentration of potassium humate from 1% to 10% respectively. After five months of respective treatment physical parameters like plant height, number of the leaves and biochemical like protein, total chlorophylls, total carbohydrates and secondary metabolites were evaluated. They were found to be in increasing order in all the treated groups than the untreated control. The maximum growth and the nutrient contents were recorded in 4% of the potassium humate treated plants than the untreated control.

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Introduction

Agriculture and agro based activities like mushroom cultivation, biofertilizer production and sericulture plays a vital role in developing countries. Among the activities sericulture is an important field in India and China. . Sericulture would fetch a handsome income to the rural woman. Thus, it would be a catalyst for development of the country. sericulture is totally dependent on mulberry (Morus alba L.) plants which are deep rooted, foliage yielding and fast growing perennial crop the leaves of the plant serve as sole food for silkworm (Bombyx mori L.) rearing. The use of mulberry as fodder will help increase the silk production from sericulture. The growth and development of the larvae and subsequent cocoon development are greatly influenced by nutritional quality of mulberry leaves (Krishnaswami, 1978). A deficiency of certain nutrients or an imbalance of nutrients in leaves causes some changes in the composition or metabolic activity of the larval body (Ito, 1972). In order to increase the nutrient content of the mulberry plants can be cultivated and treatment with various fertilizers can be imposed during cultivation. Due to reduce the cost effect of fertilizer an alternative source potassium humate was used in this study. Potassium humate contains humic acid and fulvic acid. Humic acid is organic molecule that is formed by the breakdown of organic matter. This can be obtained from coal, soil, peat and dystrophies lakes. Recently Gutierrez-Miceli et al. (2007) developed liquid formulation using vermicompost leachate having high concentration of humic acid which enhanced the growth of sorghum (Sorghum bicolor (L.) Moench). Prakash et al. (2010) have reported that Trichoderma viridi has the ability to convert lignite into humic acid. Many methods have been used to produce humic acid. The most commercial method is extraction of humic components from soft brown coal deposits with an alkaline solution. The extracted product will be alkaline pH, when the cation exchange sites are filled primarily with hydrogen (H) ions. The material is considered an acid and is called humic acid (Mikkelsen, 2005). The constituents in humic acid contain carboxyl groups and weakly acidic phenolic groups, which contribute to their complexation and ion-exchange proper-ties. They exhibit both hydrophobic and hydrophilic characteristics and can bind to soil mineral surfaces (Mikkelsen, 2005). Humic substance helps to supply nutrients to growing plants, makes soil fertile and productive, increases water holding capacity and seed germination. Humic acid also reduces the other fertilizer requirements, increases aeration of the soil, increase the protein and mineral contents of most crops (Salaman, 2005). Still very less research work has been done in the field of utilization of potassium humate in mulberry cultivation. Hence the objective of this present study was, to gain information of potassium humate, and its effects on mulberry plants and analysis of their nutrients.

Materials and methods

Potassium humate (KHA) was collected from Centre for Applied Research and Development, Neyveli Lignite Corporation Ltd, Neyveli, Tamilnadu, India. Mulberry stems of length (12 - 15 cm) were purchased from local garden (Salem district, Tamilnadu, India) were planted in the garden with 3 feet interval. Apparently, each set of these plants were subjected to spray with various concentration (1% to 10%) of dried form of KHA Potassium humate (Monthly 2 times). Finally, at the end of the every fifteen days, all the samples were collected and the growth parameters like plant height, no of leaves and nutrient factors like chlorophyll, protein, total carbohydrates, pesticidal effects and secondary metabolites were accurately evaluated by adapted methods.

Nutrients analysis

Protein:

Protein content was quantitatively measured by Lowry's method (1959). About 0.5 gm of mulberry leaf sample from apical, middle and bottom parts of the plants of different varieties was crushed and grinded in 5 ml of Trichloroacetic acid solution (T.C.A.). The grinded materials was collected in centrifugation tubes and centrifuged at 4000 rpm for 15 minutes. The clear supernatants were collected in different test tubes and assayed for protein content by

addition of Folins reagent. The solution turns blue in colour. The absorbance of blue colour was measured with the help of U.V. Spectrophotometer at 650 nm wavelength. The protein content was calculated by standard Bovine Serum Albumin. The results were expressed in mg/gm (Srivastava, 2011).

Estimation of carbohydrate contents:

Carbohydrates content in different mulberry leaves samples from apical, middle and bottom humic acid treated portion of the mulberry plant was quantitatively measured by Anthrone Reagent method (Ranganna, 1998). For estimation of carbohydrates content, 0.2 g leaves of the different mulberry varieties were grinded in distilled water with the help of mortar and pestle. Then leaves samples were centrifuged at 5000 rpm for 10 minute. The clear supernatant was collected in different test tubes and added 4 ml of Anthrone reagent was added to obtain green colour. The absorbance of green colour was taken to estimate the carbohydrate content by using U-V Spectrophotometer at 625 nm wavelength. The carbohydrates content was calculated by standard sugar solutions (Dextrose L). The results were expressed in mg/gm (Srivastava, 2011). .

Analysis of chlorophylls:

One gram of fresh leaves was weighed and minced well with scissors. 5 ml of water was added and the sample was homogenized in a blender. The final volume was made to 10 ml. an aliquot (0.5ml) was taken and mixed with 4.5 ml of 80% acetone. The acetone extracts the pigments. The supernatant was collected by centrifugation and the O.D at three wavelengths 480, 638, 645 and 663 was measured total chlorophyll content was calculated using the following formula.

Total Chlorophyll (g/lit) = (0.0202) ($O.D_{645}$) + (0.00802) ($O.D_{665}$)

Evaluation of secondary metabolites (Edeoga et al., 2005).

To identify the presence of phytochemicals in the mulberry plant the following phytochemical analysis were carried out. This evaluation is focused towards the influence of potassium humate exerts on metabolites of the mulberry plant.

Phlobatannins

1g of the extract was taken and 2 ml 1% aqueous HCL was added and boiled, formation of red precipitate which confirms the presence of phlobatannins.

Saponins

2g of the plant extract was taken with 20 ml of distilled water and few drops of olive oil were added mixed well and filtered using No: 1 Whatman filter paper.5 ml of water was added to 10 ml of the filtrate and it was shaken well. Formation of soapy forth shows the presence of saponin.

Flavonoids

Five ml of diluted ammonia was added to the aqueous filtrate of plant extract followed by addition of con sulphuric acid in drops which yields yellow colour.

Steroids

0.5 g of leaf sample was extracted in 10 ml of ethanol to the extract 2ml of anhydrous acid acid was added. Followed by 2ml of sulphuric acid, which gives violet blue colour. and confirms steroids.

Terpenoids (Salkowski Test)

5 ml of the plant extract was taken and 2ml of chloroform was added followed by 3ml of sulphuric acid. Formation of reddish brown colour shows the presence of terpenoids.

Glycosides (Keller- Kiliani Test)

2ml glacial acetic acid was added to 5 ml of plant extract with few drops of ferric chloride and 1ml of sulphuric acid. Formation of brown ring shows the presence of glycosides.

Results and discussion

In the present study, various concentrations (10%-100%) of potassium humate (KHA) were administered to the mulberry plants and the effects were observed. the maximum growth was recorded in

40% potassium humate treated plant. Prakash et al., (2011), reported that maximum growth rate of Stevia rebaudiana was recorded in 4% potassium humate treated plant. Also he reported that 60% of potassium humate enhance the mushroom growth and 0.2% of humic acid enhancing the growth of Spirulina plantensis. Potassium humate was extracted from lignite by treating with high alkali solution like sodium hydroxide and potassium hydroxide. This extracted humates contains humic acid and fulvic acid. Humic acid consist of nitrogen, potassium, phosphorus and other important plant growth promoting micro nutrients minerals. The role of humic acid is to increase the plant growth and enhance the uptake of the nutrients from the soil, also act as a chelating agent and the fulvic acid helps to transfer of the nutrient contents through the plant. In this study various parameters like i.e., physical parameters, biochemical, phytochemicals, and pest infestation effects were recorded. The leaves of the mulberry plants are the important part because they are used as sole food for the silk worms. Krishnaswami et.al (1978) observed that mulberry leaves containing water, protein total sugars, soluble carbohydrates, less minerals and crude fiber is best relished and utilized by silkworm larvae.

Table 1. Growth rate, nutrients content and pest infestation of mulberry plant.

S. No	Concentration of Potassium humate (%)	Length of the Plant (Cm) Mean±SE	No of leaves Mean±SE	Total sugars mg/g Mean±SE	Total Proteins mg/g Mean±SE	Total chlorophylls mg/L Mean±SE	Pest Infestation Mean±SE
1	1	119±0.03	72±0.02	176.0±0.03	198±0.03	20.10±0.03	15±0.04
2	2	132±0.03	88±0.03	179.5±0.04	200±0.04	20.33±0.03	15±0.04
3	3	143±0.04	102±0.04	183.2±0.04	202±0.05	20.41±0.04	14±0.03
4	4	157±0.05	107±0.05	255.3±0.05	203±0.05	20.55±0.05	11±0.02
5	5	133±0.03	100±0.04	233.1±0.04	201±0.04	20.23±0.03	13±0.03
6	6	117±0.03	80±0.02	192.2±0.04	200±0.04	10.89±0.02	14±0.04
7	7	106±0.02	63±0.01	160.1±0.03	171±0.03	10.76±0.01	14±0.03
8	8	102±0.02	55±0.01	152.0±0.03	168±0.03	10.69±0.02	13±0.03
9	9	93±0.01	44±0.01	149.3±0.04	166±0.02	10.58±0.02	12±0.02
10	10	87±0.01	40±0.01	145.0±0.04	159±0.01	10.47±0.01	Nil
11	Control	106±0.02	66±0.01	173.0±0.05	166±0.02	10.98±0.03	19±0.02

The aim of this experiment is to increase the nutrient content by treating the plant with potassium humate. Potassium humate (4%) treated plants were healthy and they are rich in nutrients like total sugars (255.3±0.05 mg/g) and control (173.0±0.05mg/g), total proteins 203±0.05 mg/g (control 1166±0.02 mg/g). Anshul srivastava (2011) reported that Karnataka- 2 middle leaves of mulberry protein content was (0.196 mg/g), TR -10 (109 mg/g), S-146 (208 mg/g) and the least value of protein content in

TR-10 (0.128mg). Disease name Pesticidal activity is important factor in mulberry plant, because infected plants may transfer to the silk warms. In order to avoid the pest infestation, the mulberry plants should more care for the growth period. In the present investigation, all the treated plants are not much infected than the control. Humic acid is not acting as biocontrol agent and pest activity; but enhancing natural plant's resistance disease and pest infection. KHA helps to increase the growth rate in terms of height of the plant; number of the leaves was monitored periodically. The maximum growth rate was obtained from 4% (Height: 157±0.05 cm, No of leaves: 107±0.05) of KHA treated plants. The total chlorophyll content in 4% treated plant contains 20.55±0.05 mg/L and control 10.98±0.03 mg/L. Potassium is a major nutrient for all the plants, which help to improve the flowering, seeds, enhance the metabolic activity and disease resistant. Similarly Shankar (1999) reported that 240 kg of K_2O/ha and 1% KCL foliar spray mulberry treated plants gave maximum yield of leaves and chlorophylls were recorded when administered to silkworm as feed also increase the cocoon production. Potassium humate is prepared from lignite by treating with KOH. Potassium humate consist many plant nutrients like nitrogen, phosphorous, potassium and some humates like fulvic acid.This nutrients directly mixed with water and spray on the plants surface. This type of treatment is easy also the nutrients directly adsorbed by the plants. Similary David (1991) reported that foliar application of Enersol (liquid humate containing 12% humic dissolved in 1 N KOH) on snap bean gave higher yield. Mann and Aspheract (1993) reported that foliar sprays of 13, 19, 26, 37 ppm vermi wash increased yield of soybean by 10-15%. Chellaiah and Gopalaswamy (1995) obtained highest yield of rice 625 kg/ha (control 382kg/ha) by applying 2% DAP + 0.5% humic acid. In this present study, we estimated phytochemical compounds like Phlobatannins, Saponins, Flavonoids, Steroids, Terpenoids, Glycosides, from treated and untreated control. From these phytochemical studies all biochemical pathways functioned as normal and were not hindered due to the administration of potassium humate.

Conclusion

The report reveals that 0.4% of Potassium humate (KHA) influences the growth rate and nutrient content of mulberry. To get a better yield, add 0.4% Potassium humate along with fertilizers.

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Robert E Pettit, organic matter, humus, humate, humic acid, Fulvic acid, and humin.

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