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Effect of Weed Extract on Forage Quality of *Medicago sativa* L.

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Abstract: ***From all forage crops, which together with meadows have a major contribution in ensuring the forage base, alfalfa crop (Medicago sativa L.) occupies a position of great importance. This plant is distinguished by its forage value, high cultivation area and high digestibility, and from the point of view of farmers and world's agricultural sciences is considered to be the "Queen of Fodder Herbs". In order to determine the quality of alfalfa, a series of classical analysis for cellulose, Neutral Detergent Fiber, Acid Detergent Fiber and Acid Detergent Lipid were performed. In the present work, Oscillatoria sp. and Spirogyra sp. were used as inoculum in pot culture of Medicago sativa. The result revealed that addition of all algal extracts can enhance forage quality in all treated plants. Statistical analysis showed that there are significant differences in % ADF, % NDF, %DDM, %DMI and RFV as compared to control.***

Keywords: Spirogyra sp.; Oscillatoria sp.; *Medicago sativa* ; %ADF; %NDF; %RFV;

1. Introduction

Alfalfa, *Medicago sativa*, also called lucerne, is a perennial flowering plant in the pea family Fabaceae cultivated as an important forage crop in many countries around the world. It superficially resembles clover, with clusters of small purple flowers followed by fruits spiralled in 2 to 3 turns containing 10-20 seeds. Alfalfa is native to warmer temperate climates. Alfalfa is usually closely associated with dairy production, which is the primary use of alfalfa. However, alfalfa is also used extensively as a horse feed, and for sheep, beef and other animals. Without alfalfa, many farms and ranches would fail. Alfalfa is one of the world's most versatile crops. It is grown in environments ranging from burning hot deserts to cool high mountain valleys. Alfalfa can grow on soil ranging from beach sands to heavy clays.(1)

On the current agricultural vision, fodder production obtained from the permanent grassland, temporary grassland and forage crops, is an integral part of agricultural land management. A fair assessment regarding the quality of forage grass originating from meadows requires an overall analysis on the data regarding the botanical composition of pastures, the nutrient and mineral content and digestibility of fodder produced . From all forage crops, which together with meadows have a major contribution in ensuring the forage base,, alfalfa crop (*Medicago sativa* L.) occupies a position of great importance. From agrobiological point of view, alfalfa gathers a number of particularities: resistance to drought and low temperatures, good revaluation of irrigation water, high capacity for regeneration after mowing, high rate of competitiveness. (2)The benefits of Algae as sources of organic matter and fertilizer nutrients have led to their use as soil conditioners for centuries. About 15 million metric tons of algal products are produced annually, a considerable portion of which is used for nutrient supplements and as bio-stimulants or biofertilizers to increase plant growth and yield. Numerous studies have revealed a wide range of beneficial effects of algal extract applications on plants, such as early seed germination and establishment, improved crop performance and yield, elevated resistance to biotic and abiotic stress, and enhanced postharvest shelf-life of perishable products.(3)

Algae products exhibit growth-stimulating activities. It promote plant growth when applied in small quantities'' and are also referred to as ''metabolic enhancers".(11) Algae components such as macro- and microelement nutrients, amino acids, vitamins, cytokinins, auxins, and abscisic acid (ABA)-like growth substances affect cellular metabolism in treated plants leading to enhanced growth and crop yield.(4)(5)

Forage and feed quality analysis is often referred to as forage and feed testing. It involves determining nutrient levels in forages and feeds and is one of the most effective feed and forage management tools. This eliminates guesswork when trying to match forage and feed supplies to animal nutrient requirements, designing supplemental feeding programs, and evaluating forage production. Although many forage producers advertise forages as "leafy," "green," or "high quality," based on visual appraisal, this can be inaccurate. Visual appraisal often does not reflect forage nutrient content. Feeds are sometimes sold without detailed nutrient composition values and/or with vague ingredient listings. Laboratory analysis is the recommended way to determine forage and feed nutrient content.

This practice can give producers an idea of the nutritional value of the feed, which in turn can drive management decisions such as whether or not to supplement and how much to supplement. Not knowing the nutritive value of a feedstuff can lead to less than optimum resource management such as not supplementing enough or spending excess money supplementing animals that do not need it. (15)(16)

2. Methodology

2.1 Algal Biomass and Growth Condition

The algae obtained from natural lake. According to its morphology and microscopic observations it is identified as

Oscillatoria sp. and Spirogyra sp. belonging to brown green and green algae respectively. Sets of 250 ml Erlenmeyer flasks were supplied with 100 ml of the appropriate growth medium (Bold Basal Medium). Each flask was inoculated with inoculum of pre-grown culture of respective algae sample. All flasks were grown under controlled laboratory conditions (temperature 25±3˚C; light) in a controlled culturing chamber under a regime of 16 h light / 8 h dark.(6)(7)

2.2 Algae Extract

The cultures were harvested and the cells washed with distilled water. Cell extracts were made by grinding the algae in distilled water with a pestle and blender. An algal suspension containing 5.0 g fresh algal material in 500 ml of distilled water is referred to as a 1% extract.(12)

2.3 Pot Method

Ten healthy seeds of alfalfa plant were then grown in 1 liter pots for 60 days. No fertilizer was applied, but soil of treated seedlings was sprayed with 200 ml of algal extract every seven day, respectively. From 30 days, after every 10 days interval studied the quality analysis parameter like %Dry matter, %ADF, %NDF, %DDM, %DMI and %RFV value.

2.4 Water & Dry Matter (DM)

Dry matter basis indicates the nutrient levels in a feed sample based on its dry matter content (i.e., excluding its water content). This is also referred to as "Dry Basis," "Dry Results" or "Moisture-free Basis." As there is considerable variation in the water content of forages, excluding the water or expressing the nutrient levels on a dry matter basis eliminates the dilution effect of the water, thereby providing the essential common basis for direct comparison of the nutrient contents across different forages.(8)(13)(14)

The water and DM content of a sample are determined by drying the plant sample from respective pots after every 10 days interval above 100^0C and measuring the water loss.(9)

% water = [(sample weight – sample weight after drying)/sample weight] x 100%
% DM = 100 - % water

2.5 VAN SOEST Fiber Analysis

A newer method for evaluating the fiber fraction of feed was developed in the 1960s by P.J. Van Soest. This system was developed because it was determined that CF did not accurately estimate the energy content of forages for ruminants. This method consists of measuring NDF and ADF fractions in forages. (17)

2.5.1 Neutral Detergent Fiber (NDF)
Neutral detergent fiber estimates the intake potential of the forage. Forages with a high NDF content are considered to be lower in quality. High levels of NDF cause forages to be eaten in lesser amounts than forages with low NDF levels. The NDF content increases with advancing maturity of forages. Neutral detergent fiber is determined by boiling the sample in a detergent solution with a pH of 7.0. The soluble portion is removed (sugars, starch, pectins, lipids, soluble carbohydrates, protein, non-protein nitrogen), and the insoluble NDF fraction remains. The NDF contains cellulose, hemicellulose, lignin, silica, and any heat-damaged protein.

2.5.2 Acid Detergent Fiber (ADF)
The ADF contains cellulose and lignin. Most laboratories use ADF to estimate the digestibility and energy value of forages. High levels of ADF cause forages to be less digestible, and have a lower energy value. Acid detergent fiber is determined by boiling the sample in an acid detergent solution. The soluble portion is removed, and the insoluble ADF fraction remains.

2.6 Values calculated from laboratory analysis

Forage Dry Matter Intake (DMI) – Forage dry matter intake for ruminants (as a % of body weight) can be estimated by the following equation:
Intake, %BW = (120 / % NDF)

Digestible Dry Matter (DDM) – Digestible DM is an estimate of the percentage of the forage that is digestible that can be estimated by the following equation:
DDM, % = 88.9 - (0.779 x % ADF)

Relative Feed Value (RFV) – Relative feed value is a measure of forage intake and energy value. It is used to compare one forage to another. Relative feed value is expressed as a percentage compared to full bloom alfalfa hay, which has a RFV of 100%. Relative feed value increases as forage quality increases. Relative feed value of a forage does not take into account the protein content of the forage, which must be evaluated separately.
RFV, % = (% DDM x % DMI)/1.29

2.3.2 Statistical analysis
Statistical analysis was performed with one way ANOVA, using software KyPlot Version 2.0 beta 13(©1997-2000 Koichi Yoshioka). Means were separated using the Least Significant Difference (LSD) test at $P<0.05$.(10)(3)

Table 2: The Effect of Algae Extract on *Medicago sativa* in Pot Culture

Pot Method	Control	Sample 1	Sample 2
% WATER	0.79 ± 0.02	0.76 ± 0.03	0.76 ± 0.02
% DM	0.20 ± 0.03	0.24 ± 0.04	0.24 ± 0.03
% ADF	0.29 ± 0.01	0.28 ± 0.01	0.27 ± 0.01
% NDF	0.39 ± 0.01	0.39 ± 0.01	0.37 ± 0.01
% DDM	0.66 ± 0.01	0.67 ± 0.01	0.67 ± 0.01
% DMI	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
% RFV	161.53	161.95	163.54

*Significant at the 0.05 level
Sample 1 = Oscillatoria sp. Sample 2 = Spirogyra sp.

Table 4: ANOVA of % DM, %ADF, %NDF, %DDM, %DMI and RFV

		SS	*DF*	*MS*	*F(CAL)*
% DM	Between Groups	0.031	2	0.002	0.365
	Within Groups	0.051	12	0.004	
	Total	0.054	14		
% WATER	Between Groups	0.031	2	0.002	0.365
	Within Groups	0.051	12	0.004	
	Total	0.054	14		
% ADF	Between Groups	0.0002	2	0.0001	0.093
	Within Groups	0.01	9	1	
	Total	0.01	11	0.0011	
% NDF	Between Groups	5.15	2	2.58	0.034
	Within Groups	0.01	9	0.001	
	Total	0.01	11		
% DDM	Between Groups	0.00012	2	6.4	0.098
	Within Groups	0.006	9	0.001	
	Total	0.006	11		
% DMI	Between Groups	3.28	2	1.64	0.034
	Within Groups	4.35	9	4.38	
	Total	4.38	11		
% RFV	Between Groups	6.95	2	3.48	
	Within Groups	2650.94	9	294.54	0.012
	Total	2657.91	11		

* Significant at the 0.05 level

3. Results and Discussion

In pot culture of lucerne plant, comparison of control and treatment plants with one way ANOVA showed that treatment groups have a significant difference in weight of fresh and dry plant sample, Acid detergent fiber, Neutral detergent fiber, Digestibility dry matter, Dry matter intake and Relative feed value as compared with control. However, effect of algal culture is not the same for all parts of plants and in different plants. In addition, effect of different algal inoculum was not the same in different plants.

The results of present study also showed that growth parameters of root such as, dry and fresh weight of plant increased significantly in treated plants. Seed treated by Spirogyra sp. extract shows good indication as compared to control. Increase in dry weight of plant in treated plants represent that the root growth was increased and as a result water and nutrition uptake to gain strength. Improvement of water and nutritional elements uptake from soil can improve total plant growth. In Quality parameter analysis, Acid Detergent fibre and Neutral Detergent fiber of this study also showed the significance difference in treated plants, whereas Spirogyra sp. showed good relative feed value as compared to other.

4. Conclusion

The review of literatures showed that the production of growth substances and vitamins by the algae may be partly responsible for the greater plant growth and yield. The capacity for biosynthesis of growth promoting substances such as auxins, amino acids, sugars and vitamins (Vitamin B12, Folic acid, Nicotinic acid and Pantothenic acid) also can enhance plant growth. The other reason that can suggest for increased plant growth by using algae extract is that, the growth of algae in soil seems to influence the physical and chemical properties of soil. The water stable aggregate significantly increase as a result of algal growth and thereby improves the physical environment of the plants. Results of this study showed that both algal species showed good quality value of treated plant as compared to control. Spirogyra showed good indication of Relative feed value as compared to Control and Oscillatoria sp. of treated plant.

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Abbreviations

SS = SUM OF SQUARE
SD = Standard deviation
Df = Degree of Freedom
S.E.M= Standard error mean
Ms = Mean square
DM = Dry matter
ADF= Acid Detergent Fiber
NDF = Neutral detergent Fiber
DDM = Digestibility Dry Matter
DMI = Dry Matter Intake
RFV = Relative feed Value

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