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EFFECT OF BIOMASS HYDROLYSATE ON SOIL AND PLANT PHENOLIC CONTENTS

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Abstract

In this work, non-edible biomass hydrolysates were used as a kind of fertilizer to investigate their influences on sandy and loamy soils. Biomass hydrolysates were extracted by subcritical water process. Three different concentration of biomass hydrolysates (1000, 2500, 4000 ppm C) were used to investigate their influences on the different soil types. The pH of soil sample was slightly neutral and alkaline for sandy and loamy soils, respectively. There were no significantly a difference on total soil nitrogen. Total carbon content increased with increasing biomass concentrations. Total water soluble phenolic contents, elements (Na, Ca and K) in bean leaves irrigated with biomass hydrolysates were determined. According to results, total water soluble phenolic contents of leaves increased in following order 40P < 25P < 10P. Significant differences were obtained in potassium and calcium contents while no considerable changes were found in sodium contents of bean leaves in different growing media.

Keywords: Kenaf, green fertilizer, kenaf hydrolysate, phenolic compound, sandy soil

1. Introduction

A fertilizer is any material of natural or synthetic origin that is applied to soils or to plant tissues to supply one or more plant nutrients which are essential for the growth of plants. This also depends on soil fertility as well as organic compounds such as humic acid, seaweed and worm castings. Soil quality is the ability of soils to perform their functions well. Although soil and water provide a large of nutrients for the plants, in some circumtancesplants may show a higher demand in nutrients uptake. . In these cases, fertilizer are needed to be supply into the soil additionally. In order to increase soil fertility, animal waste, straw and other plant residues are used as natural fertilizer for thousand years. Today, the widespread use of the chemicals containing nitrogen and phosphorus or animal fertilizers performed without showing no attention has been one of the causes of environmental pollution on soil and water (Aydemir, 1979; Barraclough et al., 1984; Bayraklı, 1990).

Biomass is a renewable energy resource, which has no any contribution to the net carbon dioxide emissions in atmosphere, and it is always present in almost all parts of the world in a great potential. Any combustible material with carbon, hydrogen, nitrogen etc. contents is considered as biomass. Vegetable, animal, forestry and industrial products, by-products and their wastes, municipal solid wastes and biosolids are the well-known biomass species. Non-edible lignocellulosic biomass materials are promising types of raw materials for fertilizer because of their abundance, sustainability and low cost. Lignocellulosic material has to be converted to sugars or other low-molecular-weight compounds. One of the methods of converting lignocellulosic biomass to the sugars is subcritical water hydrolysis process. The lignocellulosic materials hydrolysis under subcritical water condition has been started with our studies (Meryemoglu et al., 2010; Meryemoglu et al., 2014; Irmak and Ozturk, 2010; Ozturk et al., 2010; Kaya et al., 2014). This method is totally environmentally, friendly and uses water as a reaction medium. The lignocellulosic hydrolysates generate a number of by products, such as furan derivatives, phenolic compounds and carboxylic acids.

This present study aimed to investigate physical and chemical changes of soil and plant by using organic-rich biomass hydrolysates for irrigation. The use of biomass hydrolysate as a natural green fertilizer and the relationships between the evolution of plant and soil were observed how plant and soil change with increasing organic matter. This process can be carried out in particularly soils that are poor in organic matters.

The application of lignocellulosic hydrolysate fertilizer carried out an important agronomic practice for stimulating bean production. Beans are nutritional powerhouses packed with protein, fibre, B vitamins, iron, potassium, and are low in fat. Bean is in first place in the edible legumes in terms of acreage and production in the world,

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representing around 26.8 million tons per year in Europe according to FAO (FAO, 2016). Therefore, beans were used an alternative seed for sowing in this present study.

2. Materyal ve Metot

2.1. Materyal

The sandy and loam soil samples used in this study were supplied from Biology Deparment of Cukurova University. The physical and chemical properties of this soils were given Tab 1. The bean seeds were commercially supplied. Kenaf was used as lignocellulosic material obtained from Faculty of Agriculture in Cukurova University and kenaf was grounded before use. The moisture conten tand ash of kenaf samples were 8.3% and 3.4%, respectively.

Table 1. The physical and chemical properties of soils

Soils	**pH**			**C %**			**N %**		
Loamy soil	7,52	±	0,02	3,24	±	0,32	0,19	±	0,03
Sandy soil	9,31	±	0,02	0,38	±	0,11	0,11	±	0,02

2.2. Metot

2.2.1. Biomass Hydrolysis

The kenaf biomass was used as a green fertilizer. Kenaf biomass was hydrolyzed under subcritical water condition for solubilization. The details of hydrolysis process can be seen at literature (Meryemoglu et al., 2010). The total carbon content of biomass hydrolysates were determined by Apollo 9000 TOC analyzer. Three different concentration of biomass hydrolysates (4000, 2500, 1000 ppm C) were used to investigate their influences on soil and plant. KWP was also used as a fertilizer without dissolved.

2.2.2. Growth of Plants

Two hundred g sandy/loam (1:1, W/W) was added to pots. One seed was sown in each pot. 9 different medias were worked out for cultivation in this study. The information about medias and labeling were given in Tab 2. In total, there were thirty treatments, each with three replicates. Bean seeds were regularly watered until harvest. The water and biomass hydrolysate (10 cm) were less given to soil in the generative phase. In the vegetation phase, it was irrigated by increasing the amount of water and biomass hydrolysate (15 cm). The experiments were to be continued for 2 months. Soil and plant analyses were performed before and after experiments.

Table 2.The information and labelling of experimental design

Label No	**Media**	**Abbreviations**
1	Control	C
2	Plant+Water	PW
3	Kenaf+Water+Plant	KWP
4	1000 ppm C Biomass Hydrolysate	10
5	2500 ppm C Biomass Hydrolysate	25
6	4000 ppm C Biomass Hydrolysate	40
7	1000 ppm C Biomass Hydrolysate+Plant	10P
8	2500 ppm C Biomass Hydrolysate+Plant	25P
9	4000 ppm C Biomass Hydrolysate+Plant	40P

2.2.3. Soil Analyses
The total nitrogen (N%) and organic carbon content (C%) of soil were determined by using Kjeldahl methods (Duchaufour, 1970) and Anne methods (Duchaufour, 1970), respectively And also, the structure (Bouyoucos, 1951) and pH (Jackson, 1958) of soil samples were performed according to literature in triplicates.

2.2.4. Plant Analyses

2.2.4.1. Chemical Analyses
The Na, Ca and K elements of leaves were determined with ICP analysis. Before analysis, the leaves were dried at 70°C in oven for 24 h. Dried and ground samples were burned in a furnace at 550°C for 6h. The solid residue left was dissolved in 5 mL of 2 M HCl andt hen, the volume of solution was adjusted to 50 mLwith distilled water (Tokay and Yaşar, 2008).

2.2.4.2. Determination of Total Water Soluble Phenolic Contents
Total water soluble phenolics contents of leaves were determined by Folin–Ciocalteau assay (Caboni et al., 1997). Total phenolic acids were expressed as gallic acid equivalents. The 0.5 ml sample was mixed with 1 ml of 10 times diluted Folin-Ciocalteau reagent and 2 mL of saturated Na_2CO_3. The mixture was vortexed for 30 s and kept at room temperature for 30 min., and its absorbance at 765 nm was recorded using a spectrophotometer (Thermo Scientific Genesys 10S UV/Vis) (Caboni et al., 1997).

3. Results and Discussion

3.1. Evaulation of Soil Analysis
Hydrolysis experiments were performed under subcritical water for 2 hours at 250 ° C. The hydrolysis yield and total organic carbon content were found 77,3±0,25% and 5500±10,25 ppm C, respectively. The hydrolysate mainly contains water-soluble carbohydrates of kenaf and other organic compounds (Irmak and Ozturk., 2010). Hydrolysate solutions were prepared in three different concentrations and these hydrolysates were used irrigating on sandy and loamy for preliminary study. Irrigation was carried out according to the soil capacity of the soil samples. The carbon content in thes oil is the main element of the overall health of the soil. It also in soil enhances the water-holding capacity of sandy soils and improves the structural stability of loamy soil that help carbon to adhere to aggregates.

The soil pH, total nitrogen and carbon values were determined after the irrigated soil samples were dried for overnight. The pH, total nitrogen and carbon values were given Tab 3. While pH values were neutral for loamy samples, the pH values of sandy soils were alkaline. The organic carbon protects the soil from strong pH changes (URL-1, 2015). Although total carbon increased with the increasing kenaf hydrolysate concentrations, there was not much change in pH values. Soil organic carbon and nitrogen has an important role in terms of sustainable soil quality, plant production and environmental impacts (Bauer and Black, 1994; Doran and Parkin, 1994). The C / N ratio of the soil is one of its characteristic equilibrium values. The C / N ratio in soils varies between 8 and 17 (Alistair, 1979) and this ratio is a very important index for soil quality (Zhang et al., 2011). This ratio also affects the pH, nutrient accumulation and the humic substance content in the soil (Yano et al., 2000). Total nitrogen was found as 0.1-0.13% and C / N ratios were found between 28.3 and 35.90 for loamy soil irrigated with biomass hydrolysates (Tab 3). The C / N ratios of sandy soils irrigated with biomass hydrolysates varies between 4.4-6.27 as expected (Tab 3). The concentration of biomass hydrolysate has no significant effect on the total nitrogen of soils. Because of that sandy soil has low organic material, and it was decided to use the mixture of these soils.

3.2.The Effect of Biomass Hydrolysates on Soil
The results of soil with/without plant samples irrigated with biomass hydrolysates for about 2 months were as shown in Tab 4. While pH values were neutral for loamy soil samples (Tab 3.,pH: 7.54), they were alkaline in sandy soil (Tab 3., pH: 9.31). In this study, mixture of two soils was used. When Tab 4 examined, it was observed that the pH values of the mixed soil samples irrigated with hydrolysate containing dissolved organic carbon in different concentrations were the average of these two soils. The pH values of soil samples vary between 8.45-8.78.

Table 3. The pH, total nitrogen and carbon values of soil samples irrigated with biomass hydrolysates

Soil	**pH**	**C%**	**N%**	**C/N**
Loamy soil	7,52 ± 0,02	3,24 ± 0,32	0,19 ± 0,03	17,05
1000 ppm C	7,53 ± 0,01	3,68 ± 0,16	0,13 ± 0,02	28,30
2500 ppm C	7,48 ± 0,01	3,59 ± 0,22	0,10 ± 0,02	35,90
4000 ppm C	7,56 ± 0,01	4,06 ± 0,52	0,13 ± 0,01	31,23
Sandy soil	9,31 ± 0,02	0,38 ± 0,11	0,11 ± 0,02	3,45
1000 ppm C	9,51 ± 0,02	0,44 ± 0,09	0,10 ± 0,03	4,40
2500 ppm C	9,49 ± 0,01	0,69 ± 0,16	0,11 ± 0,02	6,27
4000 ppm C	9,48 ± 0,01	0,51 ± 0,12	0,10 ± 0,01	5,10

The organic material improves the physical properties of soil sand provides a suitable environment for the plants and also constitutes the main source of many plant nutrients. The organic matter content of soils should be increased to achieve high efficiency while maintaining the quality and vitality of soils. The biomass hydrolysate mainly consist of water-soluble carbohydrates of biomass. Hemicellulose and cellulose are converted into lower molecular weight components by hydrolysis of kenaf and converted into sugars, formic acids, ketons, etc. According to the calculations of soils irrigated with biomass hydrolysates for about 2 months; 1,120 g, 0.070 g and 0.028 g organic carbon were given to soil with 4000, 2500 and 1000ppm biomass hydrolysate, respectively. The results showed that the total carbon values of soil samples with/without plant increase when they irrigated with high concentrations of biomass hydrolysates (4000ppm). The C / N ratios were found to be different in soil with/without plant samples irrigated with biomass hydrolysates. This difference can be attributed to the rate of decomposition of organic matter. The C / N ratios in the soils irrigated with 2500 ppm and 4000 ppm biomass hydrolysate vary between 20-24 (Tab 4). It can be said that organic decomposition in these soils slow down but continue. The organic decomposition rate is higher in soils with a C / N ratio less than 15 (Tab 4).As a result of increasing the carbon content in soil that caused decreases in the organic decomposition rate. Also, it was observed that the concentration of biomass hydrolysate did not have a significant effect on the total nitrogen values of soils during irrigation. Well-decomposed organic matter does not provide enough nutrients to plants and soil microorganisms but they stil play an important role in the soil with their properties such as water retention, nutrient leakage, soil compaction and prevention of crusting.

In the case of plant-free soil samples irrigated with biomass hydrolysates at lower concentrations, there is a decrease in carbon values compared to the control soil sample. The carbon value of the soil did not change (1.56 %) when biomass was used without dissolution. This result showed that the kenaf did not begin to decompose in the soil during the irrigation period.

Table 4. The properties of soil with/without plant irrigated with different concentrations of biomass hydrolysate

Soils	**pH**	**C%**	**N%**	**C/N**
Control (Mixture of sandy/loamy)	8,75±0,01	1,52±0,32	0,12±0,03	12,67
Plant+water (PW)	8,78±0,02	1,62±0,21	0,10±0,02	16,20
1000 ppm C Kenaf Hydrolysate	8,48±0,00	1,08±0,02	0,12±0,03	9,00
2500 ppm C Kenaf Hydrolysate	8,45±0,01	1,20±0,25	0,05±0,01	24,00
4000 ppm C Kenaf Hydrolysate	8,55±0,01	2,14±0,44	0,11±0,01	19,45
Kenaf+water+Plant (KWP)	8,74±0,02	1,56±0,11	0,10±0,03	15,60
1000 ppm C Kenaf Hydrolysate +Plant (10P)	8,73±0,03	0,81±0,23	0,08±0,01	10,12
2500 ppm C Kenaf Hydrolysate +Plant (25P)	8,54±0,01	1,21±0,16	0,09±0,02	13,44
4000 ppm C Kenaf Hydrolysate +Plant(40P)	8,53±0,02	2,18±0,31	0,10±0,01	21,80

3.3. Determination of Total Water Soluble Phenolic Contents in Bean Leaves

The leaves of grown bean were collected and dried overnight at lower temperatures (70 °C). The 0.1 g of the leaves were grounded with blender and the 20 mL of distilled water was added. The leaves extracts were prepared at the boiling temperature of the water for 1 hour under reflux with stirring. The extracts were filtered to separate the solid particles. Total water soluble phenolics contents of bean leaves were determined by Folin–Ciocalteau assay at 765 nm (Caboni et al., 1997). Plants produce a variety of secondary products that carry a phenol group. When total water soluble phenolic content of bean leaves were examined, it was seen that total water soluble phenolic content of the bean leaves grown in soil irrigated with just water was around 0.7%. Total water soluble phenolic contents of bean leaves irrigated with biomass hydrolysates increased in following order 40P < 25P < 10P (Tab 5). Phenolic compounds effectively prevent oxidation in nutrient systems and they are as a protective factor against oxidative damage in the human body (Bartolomé et al., 1997). The total water soluble phenolic contents of the bean leaves grown in soil irrigated with biomass hydrolysates decreased in increasing biomass concentration. The maximum total water soluble phenolic content was obtained from bean leaves grown in soil irrigated with biomass hydrolysate containing 1000 ppm C. The amount of total phenolic compounds of bean leaves grown in soil irrigated with high concentration biomass hydrolysate was less than the control (Tab 5., 0.65%). It was observed that the bean leaves grown in without the use of any fertilizer were almost the same when biomass was directly used as a fertilizer. In general, total water soluble phenolic showed differences due to soil properties.

Table 5. Total water soluble phenolic contents of bean leaves

Extracts	**Total water soluble phenolics %**
Plant+Water (PW)	0,73±0,21
Kenaf+Water+Plant (KWP)	0,75±0,25
1000 ppm C KenafHydrolysate+Plant (10P)	1,06±0,32
2500 ppm C KenafHydrolysate+Plant (25P)	0,90±0,15
4000 ppm C KenafHydrolysate+Plant (40P)	0,65±0,20

3.4. Determination of Na, Ca and K in Bean Leaves

Soils consist of mineral substance, organic matter, water and air. The plant nutrients in the soil basically come from two sources. One of these sources is elements in the structure of the main material forming the soil and the other is organic and mineral fertilizers and plant residues added to the soil. The nutrients that come from these sources form the basic nutrients store in the soil (Dormaar et al., 1986). The plant continues to grow as long as it receives the nutrients it needs and amount. In other words, the growth of the plant is limited by the intake of nutrients. For this reason, it is necessary to use the nutrients in appropriate amounts and formulations together with the fertilizers from the leaves, in order to ensure the healthy development of the plants.

In this study, soils with/without plant was irrigated with organic material rich biomass hydrolysates and changes in the chemical properties of the plant were investigated. The Na, Ca and K in bean leaf samples were analyzed at ICP (Tokyo and Yaşar, 2008).

Table 6. Analysis of bean leaves

Extracts	**Nutrient Element Contents (%)**		
	Na	Ca	K
Plant+Water (PS)	0,26±0,01	3,19±0,01	0,88±0,02
Kenaf+Water+Plant (KWP)	0,12±0,02	2,38±0,02	0,62±0,01
1000 ppm C Kenaf Hydrolysate+Plant (10P)	0,18±0,02	1,40±0,01	0,75±0,01
2500 ppm C Kenaf Hydrolysate+Plant (25P)	0,11±0,01	1,29±0,01	0,77±0,01
4000 ppm C Kenaf Hydrolysate+Plant (40P)	0,16±0,01	0,10±0,01	1,56±0,01

The potassium is an essential nutrient for plants and the most abundant cation in plants. Compared to the carbon content in the soil samples and the potassium element in the leaves, it was observed that the amount of potassium in bean plant leaves grown in riched organic carbon soil samples was high (Tab 6). The amount of calcium decreased with increasing biomass concentration. Leaves with the highest amount of calcium and sodium were obtained from plants grown in water-irrigated soils. The results showed that the biomass hydrolysates used as liquid fertilizers have a positive effect on potassium, a negative effect on calcium and do not cause a significant change for sodium. Organic matter improves the physical properties of soils and provides a suitable environment for plants.

4. Conclusion

The present study was designed to hydrolyse kenaf biomass in subcritical water and use these hydrolysates a green fertilizer. Effect of different kenaf hydrolysates concentrations on soil and bean plant was investigated. The results indicated that the soil samples and plant irrigated with higher concentration biomass hydrolysate (4000 ppm C) have higher total carbon content. The C / N ratios were found to be different in soil with/without plant samples applied with biomass hydrolysates. It can be said that organic decomposition rate is higher in soils with C / N ratio less than 15. It was observed that the concentration of biomass hydrolysate had no significant effect on the total nitrogen of soil samples. The pH values of soil samples were found between 8.45-8.78. The total water soluble phenol in the leaves of beans irrigated with biomass hydrolysates decreased with increasing biomass hydrolysate concentration. The uses of biomass hydrolysates in the irrigation affect potassium and calcium elements of bean leaves grown in different media. In order to achieve high efficiency by preserving the quality and viability of soils, the organic matter content of the soils should be increased or at least the current situation should be maintained. The quality of the fruits and vegetables that grow in soils with good soil quality is also good due to the content in the organic media.

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