



Effect of Comboutea (Kombucha) and Mix Microorganisms Culture Comboutea Production Waste on Basal Respiration and Microbial Biomass Carbon Content of Soils

Comboutea (Kombucha) ve Comboutea Üretim Atıkları Karışık Mikroorganizma Kültürünün Topraklardaki Toprak Solunumu ve Mikrobiyal Biyokütle Karbon İçeriği Üzerine Etkisi

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EFFECT OF COMBOUETA (KOMBUCHA) AND MIX MICROORGANISMS CULTURE COMBOUETA PRODUCTION WASTE ON BASAL RESPIRATION AND MICROBIAL BIOMASS CARBON CONTENT OF SOİLS

ABSTRACT:

This study aims to determine the effects of comboueta and lyophilized comboueta culture on soil some soil biological features. This study was carried out on 2 different soils as a pot experiment in the greenhouse. 4 doses of comboueta (0, 10, 20, 30 ml/pot) and 4 doses of lyophilized comboueta culture (0, 0.25, 0.50, and 0.75 mg/pot) were applied on the soils with 4 kg soil in apiece pot. At the end of the experiment, basal respiration (BR) and microbial biomass carbon (MBC) analyzes were made in the soils taken from the pots. According to the analysis results, it was observed that upregulation in the amount of comboueta and lyophilized waste of comboueta culture caused an improvement of BR and MBC of soils based on the soil texture.

Keywords: *Comboueta, Microbial Biomass Carbon, Soil Respiration, Soil, Soil Biology.*



COMBOUETA (KOMBUCHA) VE COMBOUETA ÜRETİM ATIKLARI KARIŞIK MİKROORGANİZMA KÜLTÜRÜNÜN TOPRAKLARDAKİ TOPRAK SOLUNUMU VE MİKROBİYAL BİYOKÜTLE KARBON İÇERİĞİ ÜZERİNE ETKİSİ

ÖZ:

Bu çalışmanın amacı, kombu çayı ve liyofilize edilmiş kombu çayı kültürünün toprağın bazı biyolojik özellikleri üzerindeki etkilerini belirlemektir. Bu çalışma serada saksı denemesi olarak 2 farklı toprak üzerinde gerçekleştirilmiştir. 4 kg toprak bulunan saksılara 4 doz kombu çayı (0, 10, 20, 30 ml/saksı) ve 4 doz liyofilize edilmiş kombu çayı kültürü (0, 0.25, 0.50 ve 0.75 mg/saksı) uygulanmıştır. Deneme sonunda saksılardan alınan topraklarda toprak solunumu ve mikrobiyal biyokütle karbon analizleri yapılmıştır. Analiz sonuçlarına göre, kombu çayı ve liyofilize edilmiş kombu çayı kültürünün toprak tekstürüne bağlı olarak toprak solunumunu ve mikrobiyal biyokütle karbon içeriğini artırdığı belirlenmiştir.

Anahtar Kelimeler: *Kombu Çayı, Mikrobiyal Biyokütle Karbon, Toprak Solunumu, Toprak, Toprak Biyolojisi.*



1. INTRODUCTION

Soil is a living matter. The reason why soil is a living matter is the organisms that live in and on it. For the microbial population in the soil to maintain its viability, it must have suitable conditions for these living organisms. (Aşkın et al., 2004; Kızılkaya and Aşkın, 2007). The use of microorganisms plays an important role in sustainable husbandry practices, on the other hand, the increasing use of microorganisms in agriculture is evident (Malek, 1971; Kızılkaya et al., 2003; Çakmakçı et al., 2007). Soil microbial biomass and CO₂ production are widely accepted as markers of soil quality. Microorganisms provide the cycle of plant nutrients by reducing the need for chemical fertilization as far as possible, therefore, their importance in agriculture is great. For this reason, microorganisms are used as microbial fertilizers in agriculture due to their beneficial effects on development (İsmailçelebioğlu, 1969; Emtiazi et al., 2004). Microorganisms that promote plant growth, as well nitrogen fixation and phosphate solubility, promote plant growth through the synthesis of herbal hormones and vitamins, inhibiting ethylene synthesis, increasing nutrient intake and resistance to stress conditions, and decomposition of soil organic matter (Naruala et al., 2000; Saravanan et al., 2008).

Comboutea is a beverage, and it is a fermentation product composed of mushrooms, black tea, and sugar. (Marzban et al, 2015). Comboutea is a conventional beverage used in various parts of the world, mainly in Asian countries. Also, it is claimed that comfort may have many beneficial effects on human health (Murugesan et al., 2009; Marzban et al, 2015). Comboutea is a sweetened black tea which is fermented with acetic acid bacteria and yeast culture. Comboutea has a complex chemical composition and contains vitamins, organic acids, enzymes, polyphenols, and several micronutrients (Kumar and Joshi 2016; Emiljanowicz and Malinowska-Pańczyk, 2020).

Microorganisms are supreme alternatives for sustainable agriculture (Malek, 1971; Kızılkaya et al., 2004; Çakmakçı et al., 2007). Microbial activity of soil and soil fertility are closely related, so the conversion of nutrients (C, N, P, and S) from organic to mineral structure is turning through microbial biomass in the soil (Frankenberger and Dick, 1983). This study aimed to exhibit the effects of the comboutea and lyophilized waste of comboutea culture on Soil Respiration (basal respiration-CO₂ production) and Microbial Biomass Carbon of soils.

2. MATERIAL AND METHODS

2.1. Material

2.1.1. Experiment Soil

In this study, two different soil samples were used. One of the soils was from Çorlu district of Tekirdağ province (soil A) and the other soil was taken from Bafra

district of Samsun province (soil B). The methods used to determine some parameters of the soil are given in Table 1.

Table 1. Analysis applied to determine some parameter of soil samples used in the experiment (Rowell, 1996)

Analysis	Methods
Texture (% clay, silt, sand)	Bouyoucos hydrometer method
pH	1:1 (w/v) in soil: water suspension by pH-meter
EC	1:1 (w/v) in soil: water suspension by EC-meter
CaCO ₃	Scheibler calcimetric method
Organic matter	Modified Walkley-Black method
Total N	Kjeldahl method
Available P	0.5M NaHCO ₃ extraction method

2.1.2. Comboutea

Comboutea was used in 2 different forms in the trials. The first was used directly by the manufacturer in the form offered to the market. The second form was used as waste, and mixed microorganism cultures were frozen in liquid nitrogen, they were lyophilized at -80 °C for 3 days and the cell water was removed and used as cultures that show activity when combined with water.

2.2. Methods

This study was designed and conducted as a pot trial, and it was made in the greenhouse under controlled conditions. The experiment was conducted in Samsun Ondokuz Mayıs University, Department of Soil Science and Plant Nutrition research and application greenhouse. Comboutea and lyophilized comboutea culture were applied to two different soil types, both in terms of physical and chemical contents.

In the experiment, the pot was filled with 4 kg soil of oven on dry weight. Then, 4 doses of comboutea (0, 10, 20, 30 ml) and lyophilized comboutea culture (0, 0.25, 0.50, and 0.75 mg) were added on the soils. The water lost from the pots was added every day by weighing. Wheat plants were grown in test pots, and at the end of greenhouse experiment (138th day) the plants were harvested. At the end of the experiment, fresh soil samples were taken from each pot to determine the microbial biomass carbon and soil respiration of the soils.

MBC was determined by the SIR (substrate-induced respiration) method by Anderson and Domsch (1978). BR at field capacity (CO₂ production at 22 °C without the addition of glucose) was measured, as reported by Anderson (1982).

All determinations were performed in triplicate, and all values reported are averages of triplicate determinations. The routines of the SPSS 21.0 statistical program were used for the statistical analysis.

3. RESULTS AND DISCUSSION

3.1. Soil Physical and Chemical Properties

The major physical and chemical contents of the soils in the current study are presented in Table 2. Accordingly, one of the trial soils is sandy loam and acid reaction, and the other is loam and alkaline.

Table 2. Physical and chemical properties of trial soils

Soil Properties		Soil A	Soil A
Texture	Sand, %	63.03	42.15
	Silt, %	21.14	42.35
	Clay, %	15.83	15.00
	Texture Class	Sandy loam	Loam
	pH	5.80	8.01
	EC	0.13	0.10
	CaCO ₃	<1	7.5
	Organic matter	1.84	1.10
	Total N	0.11	0.10
	Available P	17.00	3.22

3.2. Properties of Comboutea

The mineral contents of comboutea were determined by Acme Labs (Acme Analytical Laboratories (Vancouver) Ltd. 1020 Cordova St. East Vancouver BC V6A 4A3 Canada in ICP-MS, and results are given in Table 3. According to the analysis results, it was determined that comboutea contains nutrients, especially P, and contains potentially toxic heavy metals (Cd, Pb...) in very traces.

Table 3. Minerals content of comboutea

Ag	< 0.5 ppb	Er	< 0.1 ppb	Nb	< 0.1 ppb	Sn	2.4 ppb
Al	5041 ppb	Eu	< 0.1 ppb	Nd	< 0.1 ppb	Sr	282.1 ppb
As	< 5 ppb	Fe	192 ppb	Ni	55 ppb	Ta	< 0.2 ppb
Au	< 0.5 ppb	Ga	< 0.5 ppb	P	3470 ppm	Tb	< 0.1 ppb
B	1421 ppb	Gd	< 0.1 ppb	Pb	4 ppb	Te	< 0.5 ppb
Ba	70.8 ppb	Ge	< 0.5 ppb	Pd	< 2 ppb	Th	< 0.5 ppb
Be	< 0.5 ppb	Hf	0.2 ppb	Pr	< 0.1 ppb	Ti	< 100 ppb
Bi	< 0.5 ppb	Hg	< 1 ppb	Pt	< 0.1 ppb	Tl	0.3 ppb
Br	68 ppb	Ho	< 0.1 ppb	Rb	352.9 ppb	Tm	< 0.1 ppb
Ca	46.6 ppb	In	< 0.1 ppb	Re	< 0.1 ppb	U	< 0.2 ppb
Cd	< 0.5 ppb	K	128 ppm	Rh	< 0.1 ppb	V	2 ppb
Ce	0.3 ppb	La	0.1 ppb	Ru	< 0.5 ppb	W	< 0.2 ppb
Cl	11 ppm	Li	9 ppb	S	12 ppm	Y	0.2 ppb
Co	0.9 ppb	Lu	0.1 ppb	Sb	0.6 ppb	Yb	< 0.1 ppb
Cr	275 ppb	Mg	18.7 ppm	Sc	18 ppb	Zn	11 ppb
Cs	1.0 ppb	Mn	4828 ppb	Se	< 5 ppb	Zr	15.2 ppb
Cu	29 ppb	Mo	2 ppb	Si	19584 ppb		
Dy	< 0.1 ppb	Na	17.6 ppm	Sm	< 0.2 ppb		

While producing comboutea, non-pathogenic bacteria and yeasts are used in the fermentation period. (Mayer et al., 1995; Mo et al., 2008; Jayabalan, 2010).

Bacteria:

Acetobacter xylinus (synonym: *Gluconacetobacter xylinus*)

Acetobacter aceti

Acetobacter pasteurianus

Yeasts:

Schizosaccharomyces pombe (synonym: *Schizosaccharomyces malidevorans*)

Saccharomycodes ludwigii

Saccharomyces cerevisiae (synonym: *Saccharomyces aceti*, *Saccharomyces capensis*, *Saccharomyces chevalieri*, *Saccharomyces hienipiensis*, *Saccharomyces italicus*, *Saccharomyces norbensis*, *Saccharomyces ole*)

Kloeckera apiculata (synonym: *Hanseniaspora uvarum*)

Zygosaccharomyces rouxii (synonym: *Kluyveromyces osmophilus*, *Saccharomyces rouxii*)

Zygosaccharomyces bailii (synonym: *Saccharomyces bailii*, *Saccharomyces elegans*)

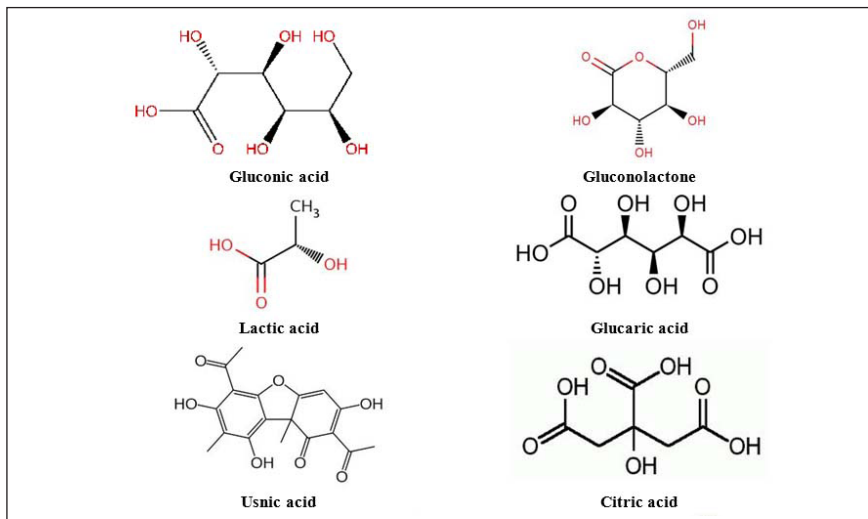
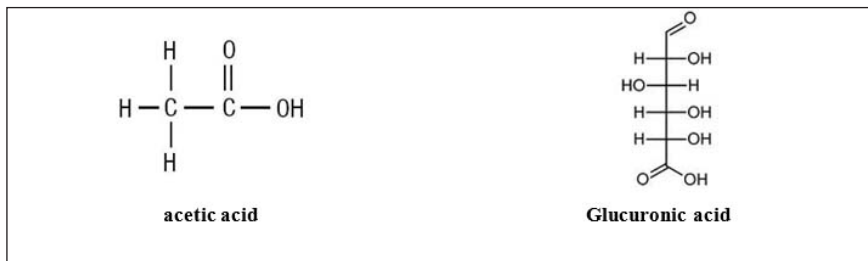
Brettanomyces bruxellensis (synonym: *Brettanomyces abstinens*, *Brettanomyces bruxellensis*, *Brettanomyces custersii*, *Brettanomyces intermedius*, *Brettanomyces lambicus*, *Dekkera intermedia*, *Dekkera bruxellensis*)

Pichia membranaefaciens (synonym: *Candida valida*, *Pichia alcoholophila*)

Candida kefyr (synonym: *Candida macedoniensis*, *Candida pseudotropicalis*, *Kluyveromyces cicerisporus*, *Kluyveromyces fragilis*, *Saccharomyces marxianus*)

Candida krusei (synonym: *Endomyces krusei*, *Saccharomyces krusei*, *Issatchenkia orientalis*)

Microorganisms (yeast and bacteria) used in the production of kombucha synthesize various organic acids, amino acids, and vitamins, it is also contained in the final product. Organic acids in the content of kombucha tea are given in figure 1, amino acids in figure 2, vitamins in figure 3, and tea polyphenols in figure 4.



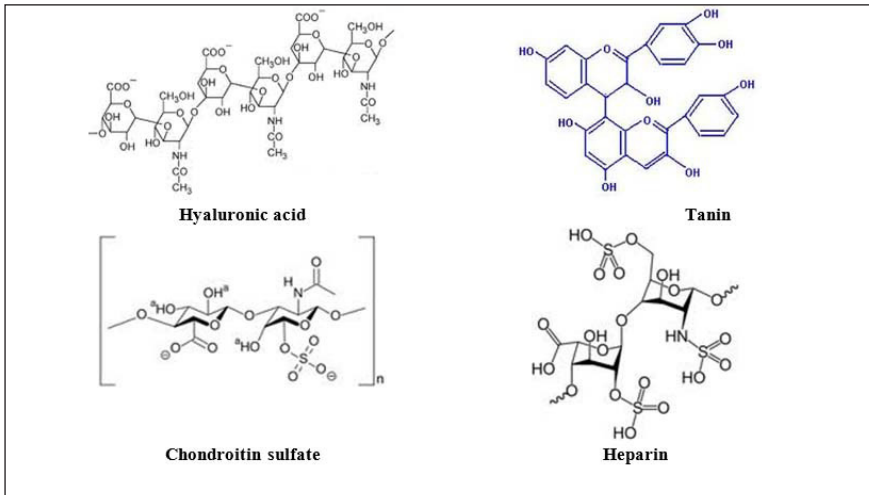
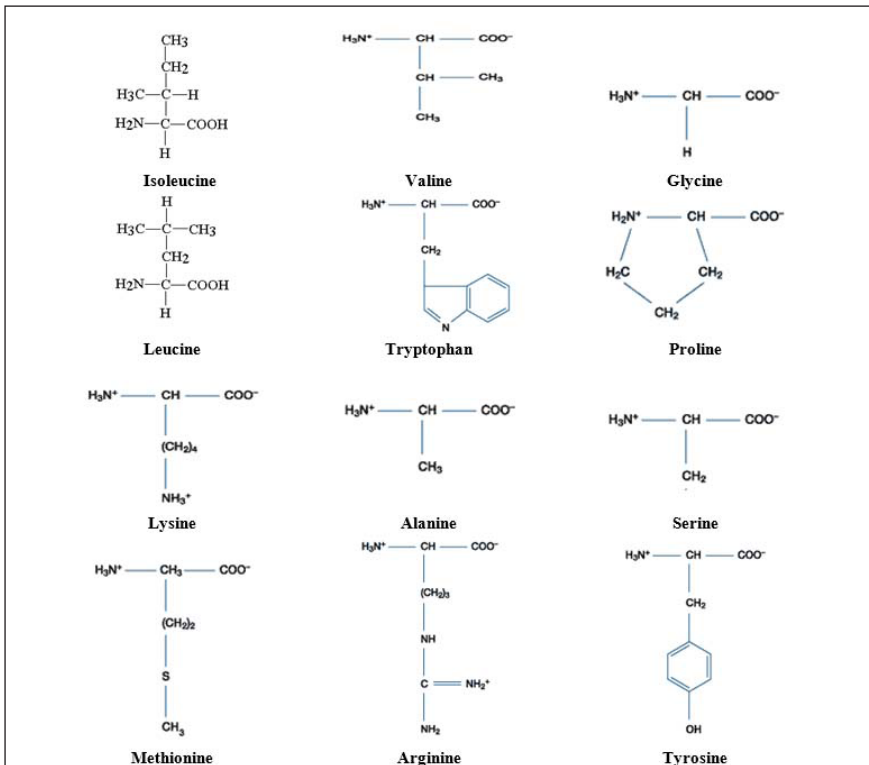


Figure 1. Various organic acids within comboutea (Blanch, 1996; Srinivasan, 1997; Dufresne and Farnworth, 2000; Sreeramulu et al. 2000; Jayabalan et al. 2007; Mo et al. 2008)



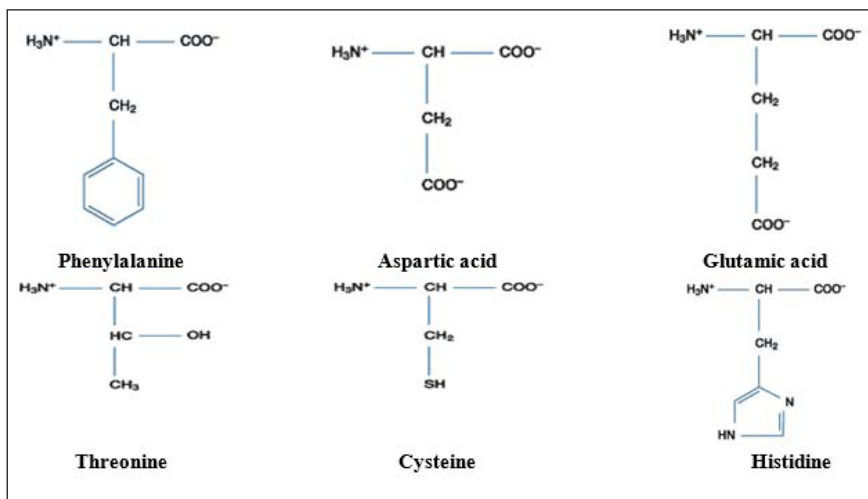


Figure 2. Various aminoacids within comboutea (Jayabalan, 2010)

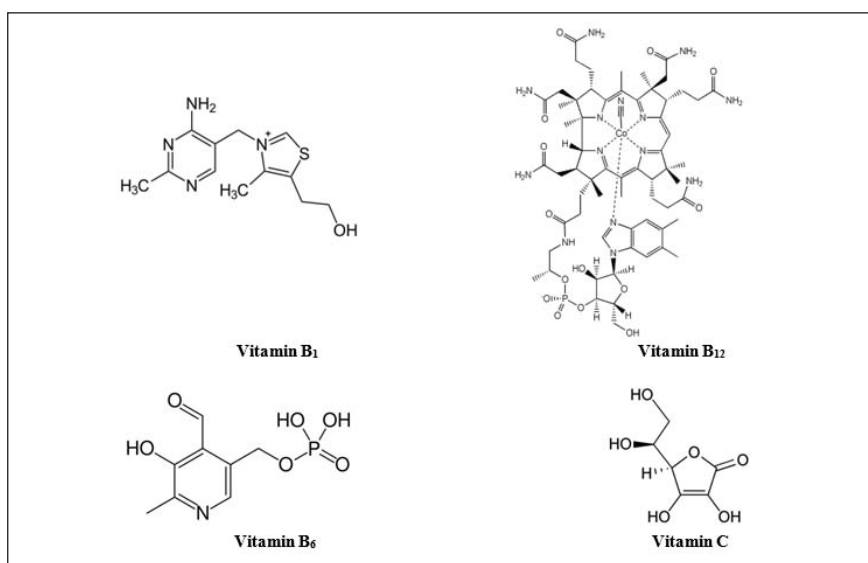


Figure 3. Various vitamins within comboutea (Bauer-Petrovska ve Petrushev-ska-Tozi, 2000)

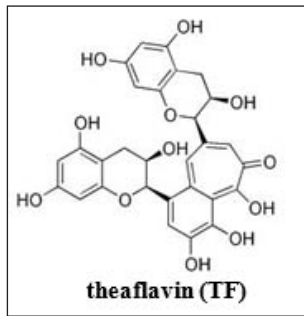
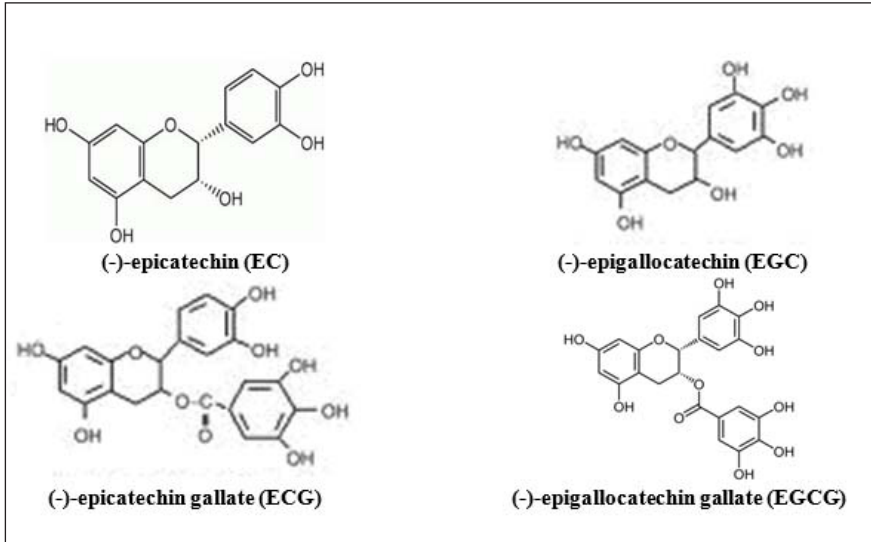


Figure 4. Various tea polyphenols within comboutea (Jayabalan, 2010)

Both the lactic acid contained in comboutea and the lactic acid synthesized by the waste microbial mixed culture used by lyophilization (Emiljanowicz and Malinowska-Pańczyk, 2020) has a very strong sterilizing feature, suppresses the population of pathogenic bacteria, and accelerates the mineralization of soil organic matter. At the same time, lactic acid prevents the reproduction and spread of *Fusarium*, which negatively affects vegetative productivity in agriculture. As a result, it increases the resistance of plants to pathogens.

3.3. Biological Properties of Soils

3.3.1. Basal Respiration (BR)

The effects of comboutea applied from the soil and lyophilized waste comboutea culture on BR in 2 different soils with sandy loam and loam are given in figure 5 and table 4. According to the results, it was determined that comboutea applied to the soil at increasing levels increased the amount of BR in both sandy loam and loam soils, depending on the increasing doses of comboutea and lyophilized waste comboutea culture dose.

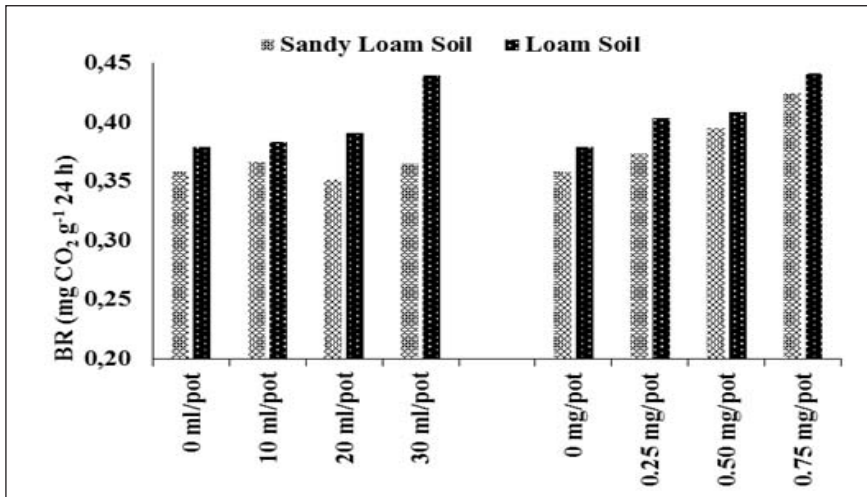


Figure 5: The effects of comboutea applied from the soil and lyophilized waste comboutea culture on soil respiration

Table 4: The effects of comboutea applied from the soil and lyophilized waste comboutea culture on soil respiration

Applications	Doses	Sandy Loam Soil	Loam Soil
Comboutea applied	0	0.358 ± 0.033	0.379 ± 0.006
	10 ml/pot	0.366 ± 0.021	0.383 ± 0.013
	20 ml/pot	0.351 ± 0.029	0.391 ± 0.013
	30 ml/pot	0.365 ± 0.019	0.439 ± 0.029
Lyophilized waste comboutea culture applied	0	0.358 ± 0.033	0.379 ± 0.006
	0.25 gr/pot	0.373 ± 0.025	0.403 ± 0.020
	0.50 gr/pot	0.395 ± 0.010	0.408 ± 0.006
	0.75 gr/pot	0.424 ± 0.011	0.441 ± 0.021

The numbers are the mean of 3 parallels and are given with standard deviations.

Variance Analysis Table and LSD Test Results						
Source	df	Sum of Square	Mean Square	F value	α type error probability	LSD (%1)
absolute frequency	2	0.001	0.001	1.676 ni	0.2028	
Soils (S)	1	0.010	0.010	24.670 ***	0.0001	0.016
application (A)	1	0.004	0.004	10.114 **	0.0037	0.016
SxA	1	0.001	0.001	2.405 ni	0.1279	-
doses (D)	3	0.015	0.005	12.510 ***	0.0001	0.023
SxD	3	0.001	0.000	0.875 ni	0.4672	-
AxD	3	0.002	0.001	1.601 ni	0.2089	-
SxAxD	3	0.002	0.001	1.757 ni	0.1757	-
Error	30	0.012	0.000			
General	47	0.049	0.001			

***P<0.001, **P<0.01, *P<0.05, ni: not important

Although the applications applied to sandy loam textured soil and loam soil give similar results on BR, it has been determined that the effect of waste kombu tea cultures by lyophilizing sandy loam to textured soil on BR is more pronounced. It was determined that 0.75 g/pot comboutea culture increased BR by 18.5% compared to control. However, when comboutea is applied directly to the soil as a product, the most effect was determined in loamy soil and 30 ml/pot application dose, the increase in BR at this dose compared to control was determined to be 15.8%.

CO₂ production which is determined in soils refers to the amount of CO₂ produced as a result of the respiration of soil creatures, and this is also called soil respiration. Soil creatures (soil fauna and microflora) produce a very large amount (2/3) of CO₂ produced in soils, and some (1/3) of it is produced by plant roots (Haktanır and Arcaç, 1997). For this reason, the determination of CO₂ production is also a frequently used evaluation method in determining the biological activity of soils (Anderson, 1982). It has been determined that the increases in soil respiration as a result of the application of both comboutea and lyophilized waste comboutea culture vary according to the soil texture. Also, it was determined that the increases in soil respiration as a result of the applications increased root respiration due to the increased plant growth. As a result of the addition of comboutea or waste lyophilized

kombu tea culture to the soils, it has been determined that microbial respiration also increases as root respiration as a result of the microorganism population in the soil. Dhull et al. (2004), observed the changes in biological activity in the soil by applying organic fertilizers and chemical fertilizers in different doses and mixtures to the soil. At the end of the study, they reported that organic fertilizer applications significantly increased soil respiration. Garcia-Gil et al. (2004), the physical (aggregation), chemical (pH, EC, total organic C and C fractions), and biological (microbial biomass C, soil respiration, dehydrogenase, phosphatase, β -glycosidase, and urease) properties were investigated. At the end of the study, they determined that aggregate stability, soil respiration, dehydrogenase, urease, and phosphatase activity were higher in soils where plants were grown compared to the control soil. Many studies show that (Hadas et al., 2004; Parfitt et al., 2005; Joergensen and Potthoff 2005; Kaur et al. 2005; Kızılkaya et al., 2007) organic origin materials applied to soils increase the amount of CO_2 production and report that the reason for this increase is narrow C:N and nutrient-rich organic materials.

3.3.2. Microbial Biomass Carbon (MBC)

The effects of comboutea applied from the soil and lyophilized waste comboutea culture on MBC in 2 different soils with sandy loam and loam are given in figure 6 and table 5. According to the results, it was determined that comboutea applied to the soil at increasing levels increased the amount of MBC in both sandy loam and loam soils, depending on the increasing doses of comboutea and lyophilized waste comboutea culture dose.

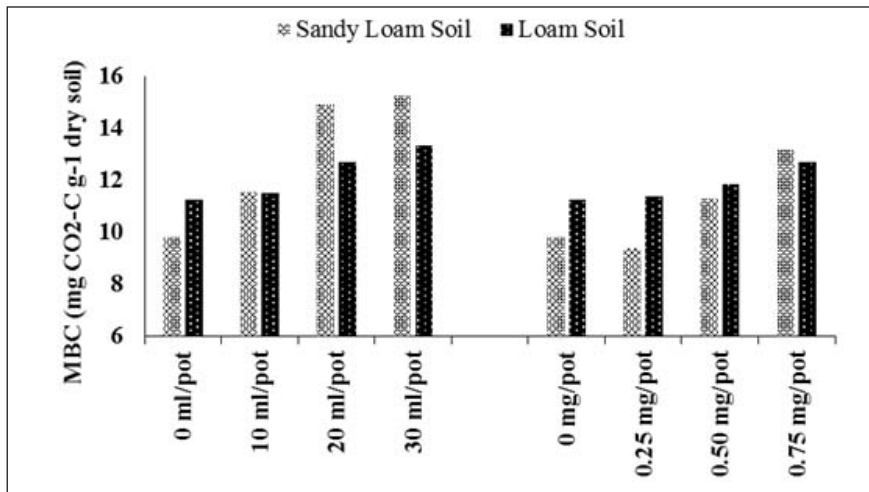


Figure 6. The effects of comboutea applied from the soil and lyophilized waste comboutea culture on microbial biomass carbon

Table 5. The effects of comboutea applied from the soil and lyophilized waste comboutea culture on microbial biomass carbon

Applications	Doses	Sandy Loam Soil	Loam Soil
Comboutea applied	0	9,817 ± 1,491	11,247 ± 0,947
	10 ml/pot	11,550 ± 0,798	11,483 ± 0,163
	20 ml/pot	14,905 ± 1,245	12,670 ± 1,166
	30 ml/pot	15,253 ± 0,454	13,308 ± 0,193
Lyophilized waste comboutea culture applied	0	9,817 ± 1,491	11,247 ± 0,947
	0,25 gr/pot	9,354 ± 0,539	11,369 ± 0,523
	0,50 gr/pot	11,280 ± 1,034	11,824 ± 0,565
	0,75 gr/pot	13,154 ± 0,770	12,711 ± 0,334

The numbers are the mean of 3 parallels and are given with standard deviations.

Variance Analysis Table and LSD Test Results						
Source	df	Sum of Square	Mean Square	F value	α type error probability	LSD (%1)
absolute frequency	2	3,029	1,514	2,031 ni	0,1472	-
Soils (S)	1	0,0106	0,106	0,143 ni	0,7080	-
application (A)	1	16,922	16,922	22,692***	0,0001	0,686
SxA	1	7,530	7,530	10,098**	0,0037	0,969
doses (D)	3	75,757	25,252	33,863**	0,0000	0,969
SxD	3	15,202	5,067	6,796**	0,0015	1,371
AxD	3	7,616	2,539	3,404*	0,0297	1,371
SxAxD	3	3,146	1,049	1,406 ni	0,2595	-
Error	30	22,371	0,746			
General	47	151,679	3,227			

***P<0.001, **P<0.01, *P<0.05, ni: not important

Even though the applications applied to sandy loam textured soil and loam soil give similar results on MBC, the effect of the applications on the sandy loam textured soil is more pronounced compared to the loamy soil. It was determined that 0.75 g/pot lyophilized waste comboutea culture in sandy loam soil increased the MBC content by 33.9% compared to the control soil. In the same soil, as a result of 30 ml/pot application of directly kombu tea, it was determined that the MBC content increased by 55.4% compared to the control soil.

The population of soil microorganisms is in a very tight relationship with the physicochemical properties of soils. Cultural activities such as organic matter or waste applications on soils can also affect the population of these microorganisms (Vekemans et al., 1989). Although the population of soil microorganisms is sufficient when similar organisms are compared with each other, this population does not make sense when the whole soil microflora is evaluated. For this reason, the determination of the biomass of microorganisms such as total microbial biomass C is one of the most frequently used parameters when evaluating the microbiological properties of soils (Rogers and Li, 1985; Vekemans et al. 1989; Nannipieri et al. 1990). Albiach et al. (2000), applied 5 different organic materials (municipal solid waste, humic acid, vermicompost, waste sludge, and sheep manure) to the garden

soil, they investigated the effects of soils on enzyme activities (dehydrogenase, alkaline phosphomonoester, phosphodiester, aryl sulfatase and urease), and microbial biomass content end of 4. and 5. years, then it was determined that all of these organic residues in general cause an increase in soil enzyme activities and microbial biomass, and the highest effect was obtained from urban solid waste, sheep manure, and waste sludge application, respectively.

Bastida et al. (2008), examined the effect of organic materials with different stabilization degrees on the size, efficiency, and structure of the microbial population in the soil. At the end of the research, they found that compost application significantly increased the microbial biomass carbon in the soil.

4. CONCLUSION

The effects of comboutea and lyophilized waste comboutea cultures applied to soils on the biological properties of soils are generally positive. However, these positive effects are generally more pronounced in sandy loam soils. The main source of the increase in soil biological properties, after the application of both comboutea tea and waste lyophilized culture on soils is directly related to the introduction of microorganisms to the soil through and the entry of some organic compounds and nutrients in the product. When evaluated the amount of carbon dioxide production, it is seen that the comboutea culture applied by lyophilization increases the CO₂ production more than the comboutea applied as a direct product. This may be due to the low pH of the comboutea product, which is not lyophilized and in liquid form, as well as the microorganisms that synthesize acid in comboutea. It may have had a negative effect on plant root development in the low pH environment.

On the other hand, microbial biomass carbon, in contrast to that of carbon dioxide production, in lyophilized comboutea application resulted in lower MBC content compared to liquid culture application in both soil types. The reason for this is the number of microorganisms populations in the lyophilized waste culture and it can be thought that the liquid culture may have a more active microorganism population.

It is clear that comboutea product, whether as liquid culture or as lyophilized waste, has a positive effect on the vitality and microorganism activities of soils. However, more detailed studies are required to reach a definitive conclusion.

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Author Contribution Rates

Design of Study (Çalışmanın Tasarlanması): MD (%50), RK (%50)

Data Acquisition (Veri Toplanması): MD (%75), RK (%25)

Data Analysis (Veri Analizi): MD (%50), RK (%50)

Writing up (Makalenin Yazımı): MD (%80), RK (%20)

Submission and Revision (Makalenin Gönderimi ve Revizyonu): MD (%90), RK (%10)

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