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EFFECT OF SOME TURKISH PROPOLIS ON THE PRODUCT QUANTITY OF *AGARICUS BISPORUS* (LANGE.) SING

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Abstract

In this study, the effect of some Turkish propolis on the product quantity of cultivated mushroom *Agaricus bisporus* (Lange.) Sing. was determined. The samples of propolis were obtained from (Bursa and Erzurum) regions of Turkey. Propolis extracts were prepared as 0.5 EEP and 2.0 EEP and sprayed on compost, compost + casing soil and compost + casing soil + 1 flush of *Agaricus bisporus* at different times of growth period under controlled laboratory conditions Propolis showed stimulatory effects on the developmental stages and some parameters of the yield. An early yield of mushrooms, rapid growing and increase of total weight of harvested basidiocarps were observed as compared to control without propolis in which primordium and basidiocarp formations showed great reduction. Chemical analysis of all the harvested mushroom that were cultivated on the product conditions with propolis were made by gas chromotography (GC)-mass spectrometry (MS).

Introduction

The nature and requirements of the cultivated mushroom are such that a variety of growing systems have to be developed. The richness of this variation is influenced by the geographic and economic environments found in the different parts of the world in which mushrooms are grown. Many factors have a profound effect on the economics of mushroom production (Gaze, 1985). Carbon and nitrogen compounds, essential elements and vitamins (Wood & Fermor, 1985) are important growth requirements of most fungi. Propolis is a type of bee product which mainly contains aliphatic acids, amino acids, aromatic acids, aromatic acid esters, aromatic aldehyde, flavones, ketones and terpenoids (Ghisalberti, 1979; Velikova *et al.*, 2001). In many investigations (Bankova *et al.*, 1995; Stangaciu, 1998; Burdock, 1998; Yuqiang *et al.*, 1999) the effects of propolis were determined as antiallergic, antimicrobial, antiparasitic, antiseptic, antimicotic, antiviral, local anestesic etc., but no studies on the effect of propolis related with edible mushrooms have been made.

In the present study, propolis was used as a growth parameter for edible mushrooms. Their effects on the product quantity of *Agaricus bisporus* (Lange.) Sing., were examined.

Material and Method

Fungal strain: Commercial strain of *Agaricus bisporus* var. U_1 was selected in the spawn laboratory of İnelli Mushroom Crop in İstanbul-Turkey. Wheat grains were used for the preparation of spawn. During spawning, the age of main culture was 7 days and the samples were stored at +4°C until use.

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Cultural conditions: Pasteurised and cooled synthetic compost comprising of wheat straw, chicken manure, urea and gypsum obtained from the Mushroom Farm of Sec in Cubuk-Ankara, Turkey were used as a substrate in which the fungus was spawned.

Compost was prepared as suggested by Günay (1995) with 1.9% N, 68-70% moisture content and pH=7.2. For spawn-running, 1 kg of spawned compost was placed in each clear 15 cmØ plastic bags. The plastic bags were kept in a growth room from spawning to casing. The growing conditions were maintained at 24-26°C, 90-95% relative humidity under dark. At the end of 12th days of vegetative growth, without ventilation from healthy fully spawn were prepared for control and for each treatment. Then the bags were opened and 2.5 cm thickness of pasteurised casing soil was spread on the spawn inoculated compost. After surroundings of the casing soil with mycelium, the temperature of the growth room was lowered to 16°C and full ventilation was supplied until basidiocarp formation. Control of the conditions in the growth room was made by means of a single computer-based environmental system. Throughout the developmental stage, control of air, compost temperature, relative humidity and CO_2 level were performed under indirect light. Harvesting of the basidiocarps began between 9-12 days after casing. The mature basidiocarps of the same size (5 cmØ) were collected. After the harvest, mushrooms were trimmed following normal commercial practices. Fresh weight yields (g) were determined for each group.

Preparation of ethanol extract of propolis (EEP): The propolis was obtained from Bursa and Erzurum cities of Turkey in 1991. Propolis extracts were prepared as 0.5 EEP and 2.0 EEP concentrations. For the preparation of propolis extracts about 8.96 ml and 6.7 ml propolis was taken from stock 1 propolis respectively and added to 1.04 ml and 3.3 ml ethyl alcohol (99 %) respectively. In this way, stock 2 propolis extracts were obtained as 10 ml. For each group, 0.5 ml and 2.0 ml propolis solution were taken from stock 2 and made upto 100 ml with distilled water (Sorkun *et al.*, 1996).

Propolis application: Propolis at concentration of 0.5 EEP and 2.0 EEP was applied as a mixture to compost and casing soil by spraying to first flush. The propolis application for various group is presented in Table 1.

Table 1. Propolis application for various experimental groups of *Agaricus bisporus*.

Groups and numbers	Compost	Compost + Casing soil	1st flush
(1) Control	-	-	-
(2) 0.5 EEP Bursa	+	-	-
(3) 0.5 EEP Bursa	+	+	-
(4) 0.5 EEP Bursa	+	+	++*
(5) 2.0 EEP Bursa	+	-	-
(6) 2.0 EEP Bursa	+	+	-
(7) 2.0 EEP Bursa	+	+	++*
(8) 0.5 EEP Erzurum	+	-	-
(9) 0.5 EEP Erzurum	+	+	-
(10) 0.5 EEP Erzurum	+	+	++*
(11) 2.0 EEP Erzurum	+	-	-
(12) 2.0 EEP Erzurum	+	+	-
(13) 2.0 EEP Erzurum	+	+	++*

+ = Numbers of propolis application

- = No application

* = The interval between the application of propolis was 7 days

Statistical analysis: Anova was made and the data were calculated at 5% (LSD).

Results and Discussion

Chemical composition of used propolis: The propolis of Bursa was collected from areas where flora consists of local plants. Propolis from Erzurum region was collected mainly from grasstress especially *Astragalus* spp., and some species of *Fabaceae* and *Asteraceae*. The chemical composition of used propolis showed that the quantity of the flavones (37.55%) and aromatic acids (18.15%) are very high in the propolis of Bursa (Table 2). On the other hand the propolis of Erzurum contains highest alcohol content (21.73%) and amino acids (4.46%) but terpenoid contents of these samples is very low (Table 2).

Table 2. The chemical composition of used propolis.

Compound	Bursa (%)	Erzurum (%)
Alcohols	6.34	21.73
Aliphatic acids	6.41	1.96
Amino acids	very low	very low
Aromatic acid esters	3.10	4.46
Aromatic acids	18.15	31.86
Aromatic aldehyde	1.86	1.32
Flavones	37.55	4.72
Ketones	6.95	8.19
Terpenoids	1.84	3.31
Others	10.09	22.45

The development of mycelium on the compost and the casing soil: In the groups with propolis which had only compost, both compost and casing soil, the colonisation of mycelium were completed at shorter time than control group. The spread of mycelium was more rapid and dense and the development of mycelium was completed within 5-6 days, as compared with the control group without propolis.

The effect of propolis at the level of primordium and harvesting: In all treatments with propolis, the primordium was observed on 7^{th} day after the casing. The early initiation was the determinative feature of these groups at the beginning of harvest.

In the 0.5 EEP Erzurum Compost and 0.5 EEP Erzurum Compost+Casing soil groups the mushrooms were collected after 13^{th} day from the casing. The harvested mushrooms in the first week of harvest and after 15 days from casing showed more rapid development. The effect of propolis continued at the 2^{nd} and 3^{rd} weeks with higher yield in all groups with propolis as compared to control group where the development of mycelium was weaker and the number of fructification was very low. In this group, the features of growth in the stage of fructification showed contamination, with late and fewer yield. In the control group, 4 flushes were observed and the interval between the flushes were 10 days while all groups with propolis gave an harvest of 5 flushes and the interval between the flushes were 6 days.

The quantity of total product and the number of total fructification is shown in Table 3.

Table 3. The quantity of total product and the number of total fructification of survey groups.

Group number	Quantity of total product (g)	Number of total fructification (unit)
1	881	59
2	1551	100
3	1715	103
4	1416	92
5	1279	84
6	1450	95
7	1140	72
8	1205	80
9	1657	102
10	1113	74
11	1256	85
12	1466	95
13	1136	75

Maximum product was obtained in the group of 3 (0.5 EEP Bursa compost + casing soil) in which propolis was applied to compost and casing soil. In contrast the least product was obtained in the group of 1 (control group) which had no propolis treatment. Maximum number of fructification was obtained in the group of 3 with 0.5 EEP Bursa compost + casing soil. In all groups the numbers of fructifications are higher than the control group.

The number of fructifications increased up to 122% and 181% in all the groups respectively in comparison to the control group. The quantity of product also increased up to 126% and 195% in all the groups as compared to the control group which had no propolis treatment (Table 4 and 5).

Chemical analysis of harvested mushrooms: Preparation Ethanol extracts of Bursa and Erzurum region propolis and determination of their chemical composition has been given in detail in a previous report (Sorkun *et al.*, 2001). Ethanol extracts of these propolis were used in the present study to improve the yield of mushrooms and the production time. After application of propolis to mushroom either in compost or compost + casing soil, the mushroom samples were extracted and the extracted samples of all harvested mushroom were analysed by GC-MS system.

The result showed that in the group of 0.5 EEP Erzurum compost + casing soil + 1st flush, propolis passed to fructifications in which propolis applications were applied three times. The rate of passing propolis was calculated as 96.7% on the fructifications. The results of chemical analysis are shown in Fig. 1.

Gas chromotogram of 0.5 EEP Erzurum compost+casing soil+1st flush propolis sample contained mainly alcohol, aromatic acids, aromatic acid esters and less amount of terpenoids. This observation showed that alcohol, aromatic acids and their esters are very important on the mushroom production and the increase in mass production of mushroom is the highest when this propolis mixture is applied to the mushroom sample. This is because of the high content of alcohol (21.73%), aromatic acids (1.32%) and their esters (31.86%) in Erzurum propolis.

Statistical analysis: Anova was made and the data were calculated at 5% (LSD).

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The quantity of total product and the number of total fructification is shown in Table 3.

Table 4. The quantity of product of all the groups.

Group Number	The quantity of product (g)				
	1st week	2nd week	3rd week	4th week	5th week
1	0±0.00	185±0.57	350±0.33	230±0.88	116±0.57
2	241±0.57	332±0.57	558±0.57	285±0.57	135±0.33
3	262±0.57	371±0.33	605±0.33	302±0.33	175±0.57
4	185±0.33	322±0.57	501±0.66	242±0.57	166±0.33
5	175±0.33	295±0.57	460±0.57	213±0.57	135±0.33
6	190±0.33	330±0.57	495±0.57	255±0.57	180±0.57
7	145±0.88	275±0.57	385±0.88	220±0.57	115±0.88
8	135±0.57	280±0.88	440±1.15	215±0.57	145±1.15
9	250±1.45	365±0.33	590±0.57	290±0.57	162±1.20
10	105±0.57	272±0.57	405±0.88	203±0.88	127±0.57
11	145±0.57	290±0.57	450±0.57	225±0.57	146±0.67
12	195±0.57	340±0.57	510±0.33	250±0.57	171±1.20
13	118±0.88	281±0.57	399±0.88	208±1.20	130±0.57

LSD (5) %=1.73

Table 5. The number of fructification of all the groups.

Group Number	The number of fructification (unit)				
	1st week	2nd week	3rd week	4th week	5th week
1	0±0.00	12±0.33	23±0.58	16±0.58	8±0.58
2	15±0.58	22±0.58	37±1.00	17±0.58	9±0.00
3	16±0.33	24±0.33	41±0.33	20±0.58	12±0.33
4	12±0.33	21±0.33	32±0.33	16±0.33	11±0.33
5	12±0.33	17±0.33	32±0.33	13±0.33	10±0.33
6	12±0.00	22±0.58	32±0.33	15±0.67	12±0.33
7	10±0.33	17±0.67	25±1.00	12±0.33	8±0.33
8	9±0.33	17±0.67	30±0.33	14±0.33	10±0.67
9	15±0.66	24±0.33	34±0.33	18±0.88	11±0.33
10	7±0.33	17±0.67	27±0.33	14±0.66	9±0.33
11	10±0.66	19±0.33	31±0.33	15±0.67	10±0.33
12	13±0.33	22±0.33	34±0.33	15±0.33	11±0.33
13	8±0.33	17±0.66	27±0.67	14±0.33	9±0.33

LSD (5)% = 1.14

In the group of 2.0 EEP Erzurum compost+casing soil, propolis passed to fructifications where propolis applications were applied two times. The rate of passing propolis was 82.4% on the fructifications. The results of chemical analysis are shown in Fig. 2.

At the group of 2.0 EEP Erzurum compost + casing soil + 1st flush, propolis passed to fructifications where propolis applications were applied three times. The rate of passing propolis was 98.61% on the fructifications. The results of chemical analysis are shown in Fig. 3.

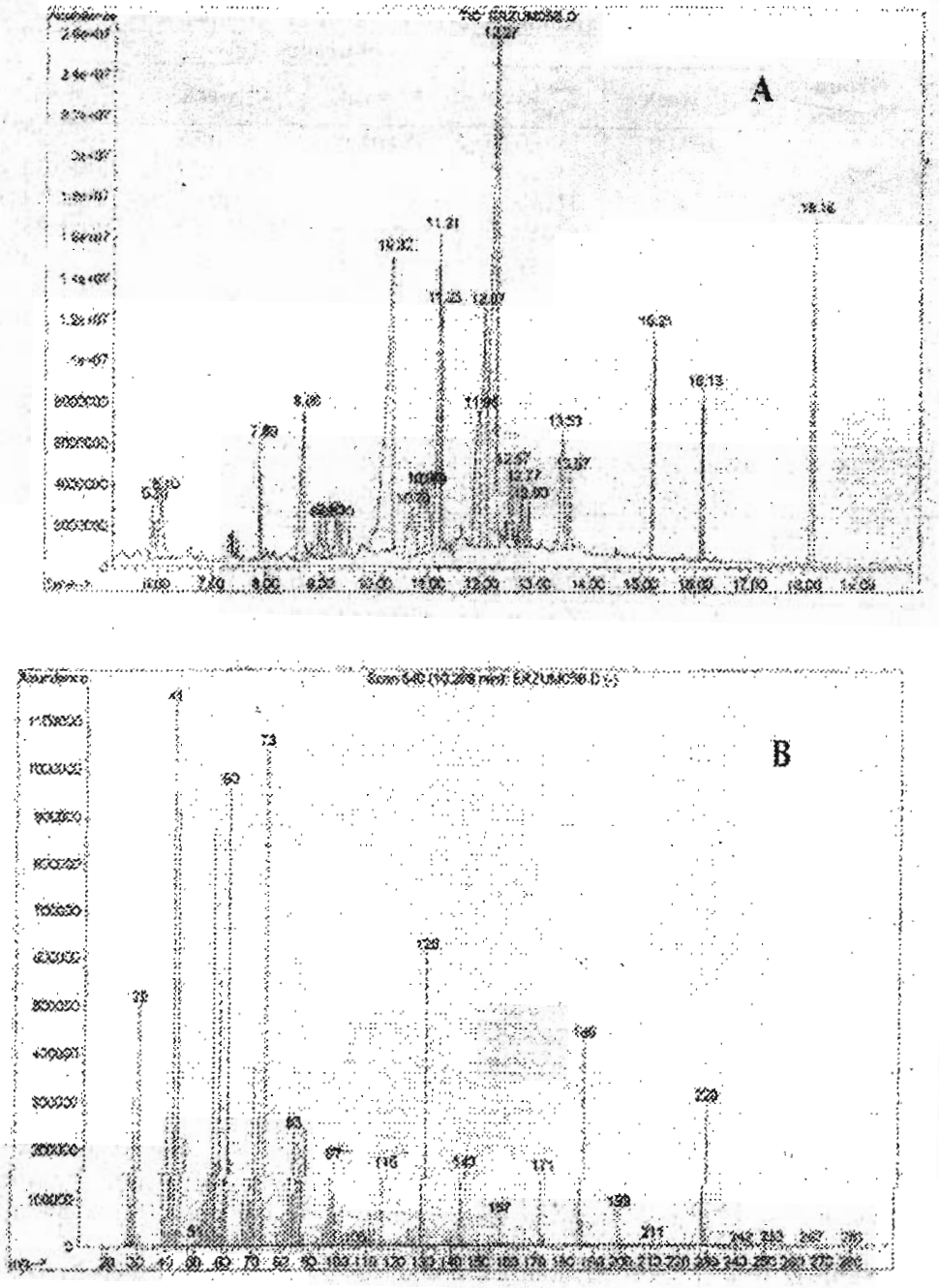

Fig. 1. The chemical analysis of group of 0.5 EEP Erzurum compost + casing soil+1st flush. (A-) Gas chromatogram of the sample, (B-) Mass spectrum of a component of the sample at 10.32 min. retention time. This component characterises a kind of carboxylic acid in the sample.

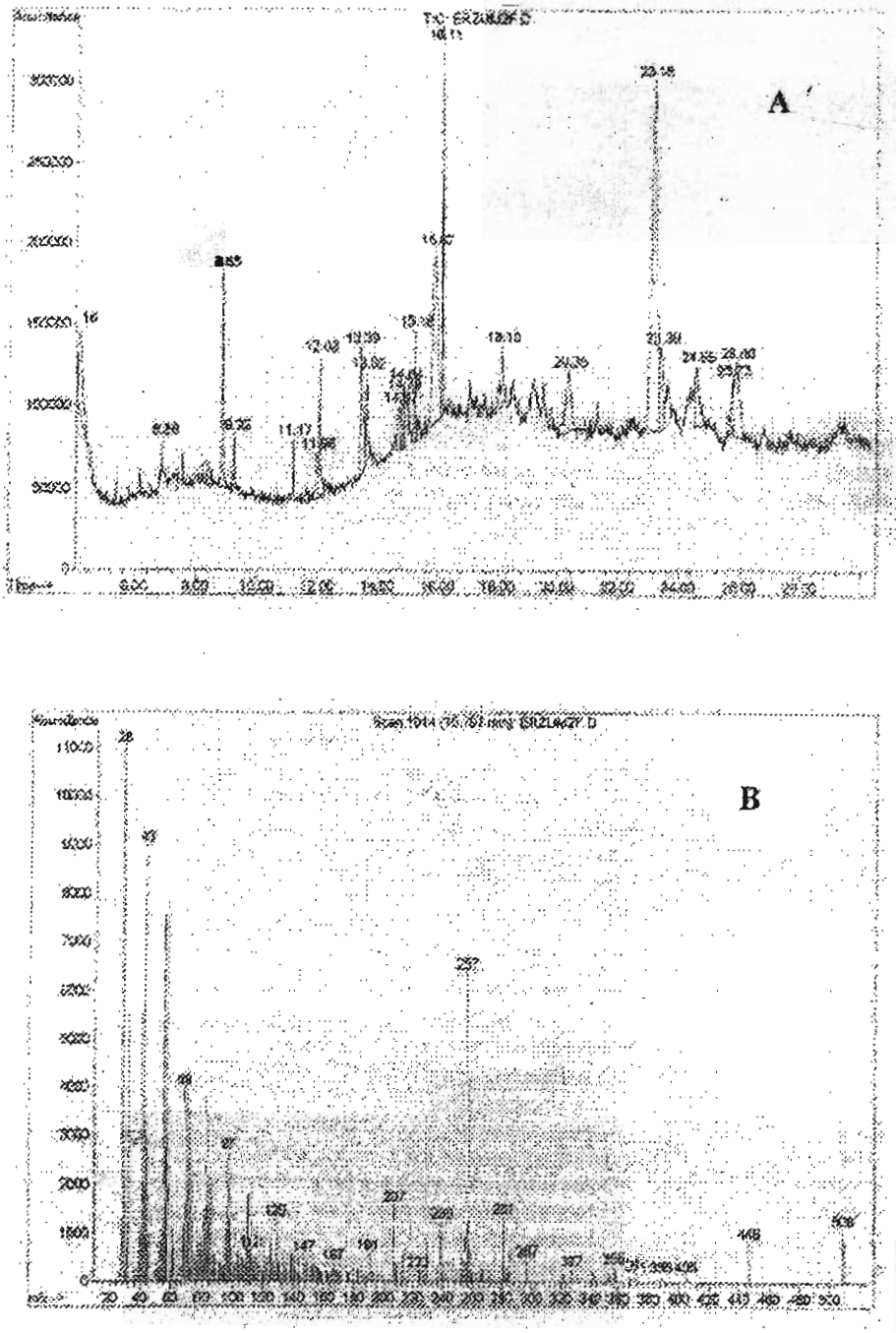

Fig. 2. The chemical analysis of group of 2.0 EBP Erzurum compost + casing soil.
A- Gas chromatogram of the sample, B- Mass spectrum of a component of the sample at 15.87 min., retention time. This component characterises a kind of flavone in the sample.

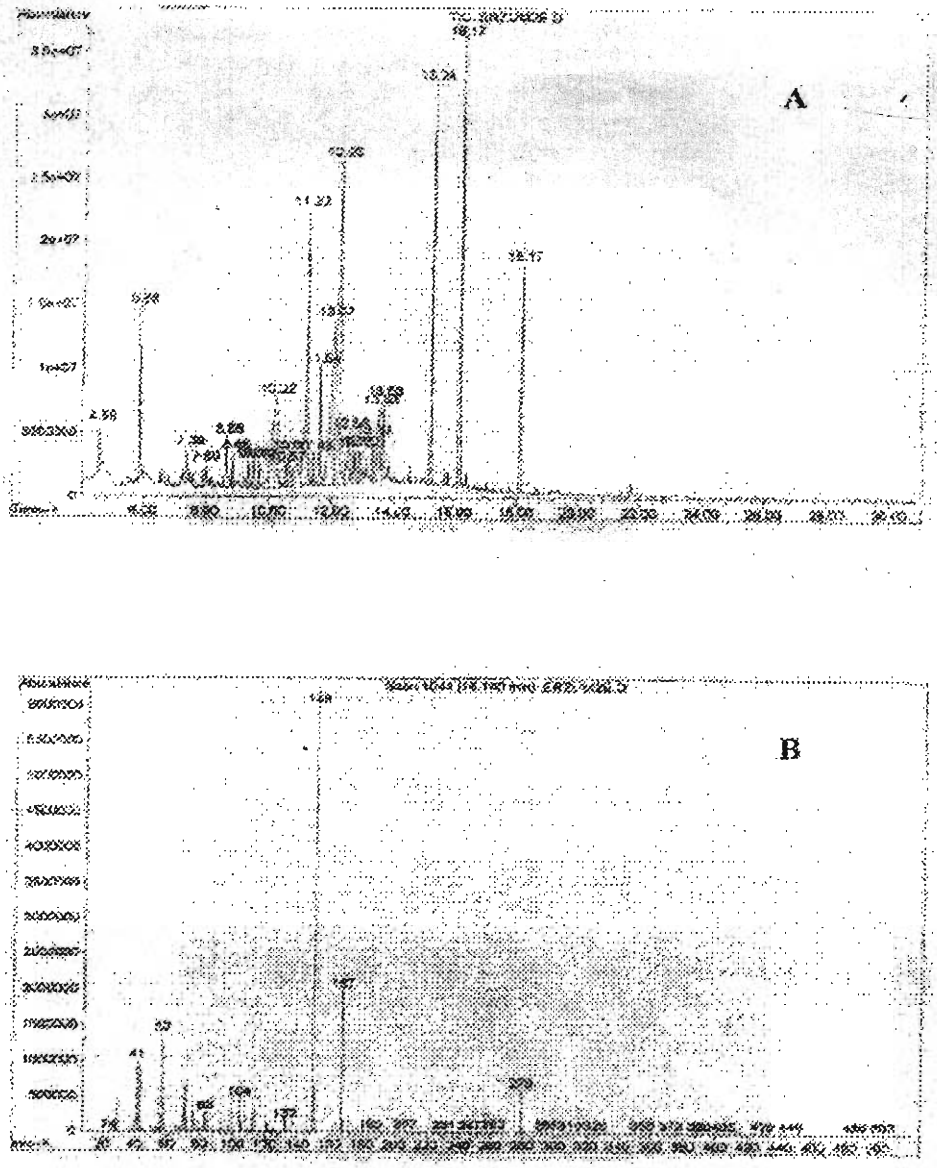

Fig. 3. The chemical analysis of group of 2.0 EEP Erzurum compost + casing soil + 1st flush.
A- Gas chromatogram of the sample, B- Mass spectrum of a component of the sample at 16.17 min., retention time. This component characterises a kind of aromatic carboxylic acid ester in the sample.

In all groups of Bursa (both 0.5 EEP and 2.0 EEP); propolis were not found in the fructifications. In these groups, the rate of propolis was found between 0.19 and 12.6% and the rates were not important.

The result of chemical analysis in the group of 0.5 EEP Erzurum compost + casing soil + 1st flush, in the group of 2.0 EEP Erzurum compost + casing soil and in the group of 2.0 EEP Erzurum compost + casing soil + 1st flush, propolis passed to fructifications. On the other hand in the control group and in all groups of Bursa (both 0.5 EEP and 2.0 EEP), propolis were not found in the fructifications.

Arkan *et al.*, (1997) reported that the development of mycelium was not obtained at 2.5, 5.0 and 7.5 EEP. It is interesting to note that studies of propolis related with edible mushrooms have not been previously reported. All these results mentioned above support directly or indirectly our results obtained with *Agaricus bisporus.*

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