Insecticidal Activity of Fatty Acid-Rich Turkish Bryophyte Extracts Against *Sitophilus granarius* (Coleoptera: Curculionidae)

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Abstract: The composition of fatty acids and insecticidal effects was performed for the Turkish mosses *Dicranum scoparium*, *Hypnum cupressiforme*, *Polytrichastrum formosum*, *Homalothecium lutescens* and the Turkish liverwort *Conocephalum conicum*. All structures were determined by means of gas chromatography and gas chromatography-mass spectrometry techniques. The determination of fatty acids was done using a simple and mild method that utilized different solvent extractions ranging from nonpolar to polar solvents (hexane, dichloromethane, chloroform, ethyl acetate and methanol, respectively), and the samples were powdered with and without liquid nitrogen. The correlations between the saturated and unsaturated fatty acid contents depending on the solvent polarity and their crushing process by liquid nitrogen were observed. The insecticidal activity of the bryophytes was analyzed by using the methanol, hexane and esterified methanol extracts. The hexane extracts of *Polytrichastrum formosum* showed the highest insecticidal activity (70.33%) against *Sitophilus granarius*. Contact toxicity activities of lauric, myristic and palmitic acids besides single dose studies of the solvent extracts were carried out. The highest mortality rate (53.34%) was obtained from the myristic acid among the tested pure fatty acids. The activities of palmitic and lauric acids were 17.75% and 4.32%, respectively.

Keywords: Fatty acids, GS-MS, insecticidal activities, liverworts, mosses, plant extracts.

INTRODUCTION

Bryophytes are among the largest groups of spore-producing land plants [1] and are found in all ecosystems (from desert to alpine) except marine. Also, the biomass of bryophytes varies in each ecosystem [2]. They are comprised of 15.000-25.000 species and are the second largest group after flowering plants (350.000 species) worldwide. Most systematists classify these plant groups as Anthocerotophyta (also known as the class hornworts), Marchantiophyta (Hepaticae) and Bryophyta (Musci) according to the recent genetic information [3]. In the bryophyte studies done up to now, these plants were used ethnobryologically and as medicinal plants in different countries. It is generally reported that many bryophyte species have been used in traditional Chinese medicine and by Native North Americans [4]. Although the systematic of bryophytes has been studied intensivelytheir chemistry is poorly known, and the literature reports on this subject are very scattered [1, 2, 5]. Mosses and liverworts have terpenoids, phenolics, glycosides, fatty acids (FAs) and aromatic compounds [2]. Liverworts are mainly preferred among bryophytes, and the bryophyte class is very interesting for chemical analysis due to its structure containing oil bodies as well as its production of a number of lipophilic terpenoids, aromatic compounds, acetogenins, etc. [2, 6, 7]. However, mosses and hornworts' not having oil bodies make them prefererred less [8].

FAs widely distributed in nature are important for organisms. They have important roles of physiological and biological functions in their life cycles [1, 5, 9]. The most commonly found FAs in bryophytes are also abundant in most of the other organisms. However, arachidonic and eicosapentaenoic acids are not found very abundantly in the rest of the plant kingdom [10]. Besides, these FAs are valuable for the leaf structure, plant size and habitat preference of the moss family members, such as in Dicranaceae family [11]. Some known FAs such as linoleic acid [12], oleic acid [13, 14], linolenic acid [15, 16], and lastly linoleic, palmitic and stearic acids [17] have shown insecticidal activity against some insects [18]. Also, Khan and Usman [19] determined the insecticidal activity of the FAs against *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.) and *Callosobruchus analis* Fabricius and the results showed that the FAs exhibited moderate insecticidal activity against *C. analis*.

Today, the heavy usage of chemical insecticides causes insects to develop resistance to them. Therefore, the studies on eco-friendly natural products have increased in order to reduce the negative effects of insect resistance. The chloroform, methanol:chloroform (1:1) and methanol extracts obtained from mosses were found to be suitable for the protection of stored products [20]. Therefore, some bryophytes contain compounds that are easily extracted and would serve as effective insecticides. As an alternative

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agricultural industry, the bryophytes are being utilized to establish cell cultures for the extract. Although many commercially useful effects of bryophytes are known, there is limited research in industrial crop protection especially in mosses and liverworts [21]. The present paper reports an analysis of the FAs of some Turkish mosses (*Dicranum scoparium*, *Hypnum cupressiforme*, *Polytrichastrum formosum*, *Homalothecium lutescens*) and the liverwort *Conocephalum conicum* belonging to different families sampled in northern parts of Turkey. In addition, the contact activities of the bryophytes were examined using methanol, hexane and esterified methanol extracts against *Sitophilus granarius*.

MATERIAL AND METHODS

Standarts and Reagents

Hexane, dichloromethane, chloroform, ethylacetate, methanol and KOH were purchased from Merck (Darmstadt, Germany). Supelco 37 standart FAME mix was purchased from Sigma Aldrich.

Plant Materials

The plant materials were gathered from different regions of Turkey. The mosses *D. scoparium* (Dicranaceae) and *H. cupressiforme* (Hypnaceae) were collected from Ilgaz Mountain National Park, located in the transitional zone between the Central Anatolia and West Black Sea Region, at an altitude of 1720 m, latitude 41° 03' 19.253" N, longitude 33° 44' 23.859" E, in September 2011. The species were collected at unpolluted sites located at approximately 100-150 (m) from the main road and more from soil and less from rocks. The woodland habitats were dominated by pure Uludağ fir (*Abiesnordmanniana* (Stev.) Spach. subsp. *bornmulleriana* (Mattf.) Coode et Cullen), and also contained a mixture of Scotch pine (*Pinus sylvestris* L.). *H. lutescens* (Brachytheciaceae) was collected from Eldivan town belonging to Çankırı province under black pine trees (*Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe) and from some places covered with *Juniperus oxycedrus* L. and *Berberis vulgaris* L., on soil, at an altitude of 1048 m, latitude 40° 30' 46.751" N, longitude 33° 30' 12.670" E, in March 2011. *P. formosum* (Polytrichaceae) and *C. conicum* (Conocephalaceae) were collected in 2011 from north-east Turkey, Fındıklı (Rize), Kıyıcık village. *P. formosum* was picked up near a tea garden on north slopes, on soil, at an altitude of 20 m, latitude 41° 19' 18.803" N, longitude 41° 14' 45.846" E, in August 2011. *C. conicum* was also gathered on wet rocks in a shady environment near a stream among alder (*Alnusglutinosa* (L.) Gaertn. subsp. *barbata* (C.A. Mey.) Yalt.) and common hazelnut trees (*Corylus avellana* L.), at an altitude of 33 m, latitude 41° 19'20.112" N, longitude 41° 14' 48.558" E, in September 2011. All the mosses and liverwort species were homogenized in the laboratory. After drying the samples, their taxonomic identification was done depending on flora books related to bryophytes [22, 23]. The voucher bryophyte samples (*D. scoparium*: ABAY 1628; *H. cupressiforme*: ABAY 1629; *H. lutescens*: ABAY 1630; *P. formosum*: ABAY 1631, and *C. conicum*: ABAY 1632) were kept in the bryophyte herbarium of ABAY in the Forest Botany Department, Çankırı Karatekin University.

Extraction Procedure

Some plant materials (5 g, each sample) of *D. scoparium, H. lutescens, H. cupressiforme, P. formosum* and *C. conicum* were crushed in liquid nitrogen and others were crushed into small pieces in air-dried conditions. The bryophyte samples were extracted respectively with hexane, dichloromethane, chloroform, ethyl acetate and methanol (220 ml), respectively, for a week for each solvent (Fig. **1**). All extracts were esterified to determine FAs with methoxide. Each extract was filtered with quantitive filter paper and evaporated under reduced pressure at 25°C. The crude extracts were dissolved in 2 ml methanol and 10 ml hexane. Then, 5 ml KOH solution (1 M) in methanol was added to the crude extract solution. The final solution was vortexed at 2500 rpm for 30 seconds. The esterified FAs were filtered and performed to GC-MS.

GC Analysis

Official methods were used to determine FAs. FA methyl esters were prepared by KOMe-catalysed trans-esterification and analyzed by GC-MS on a Agilent Technologies GC 7890A equipped with DB-WAXETR capillary column (60 m x 320 µm x 0.25 µm), filled with polyethylene glycol, at an ionization voltage of 70 eV. Helium was the carrier gas (1 mL / min^{-1}). The temperature was kept at 120 °C for 8 min, then increased to150 °C at a 8 °C min^{-1} rate, and held for 2 min, then 8 °C /min to 200 °C for 10 min, then 8 °C/min to 250 °C for 35 min, total run time 71.25 min. Injection volume was 1 µL at splitless mode. The FAs were identified by Supelco 37 standard FAME mix using the mixed FAs standards available in our laboratory. Famedb23, Famdbwax and NIST05a were employed to identify the structure of compounds by comparing with those in the library.

GC-MS Analysis

GC-MS analyses were performed on an Agilent Technologies GC 7890A with equipped 5975 Triple Axis Detector mass spectrometer. For GC-MS detection, DB-WAXETR column (60 m x 320 µm x 0.25 µm), electron ionization system and ionization energy of 70 eV were used. Helium was the carrier gas at a flow rate of 1 ml/min. The column temperate was operated under the same conditions as described above.

Insect Culture

S. granarius stock cultures were grown in a laboratory at 27±2 ºC and 60±5 rh in a dark climate chamber in the Department of Biology, at Çankırı Karatekin University. Wheat was the food medium for *S. granarius* and 1-2 month-old adults were used in trials. Tests were also carried out under the same conditions and in the same laboratory [20].

Bioassay

Methanol, hexane and esterified extracts of five bryophyte species were diluted with acetone to 50 µg/µl and

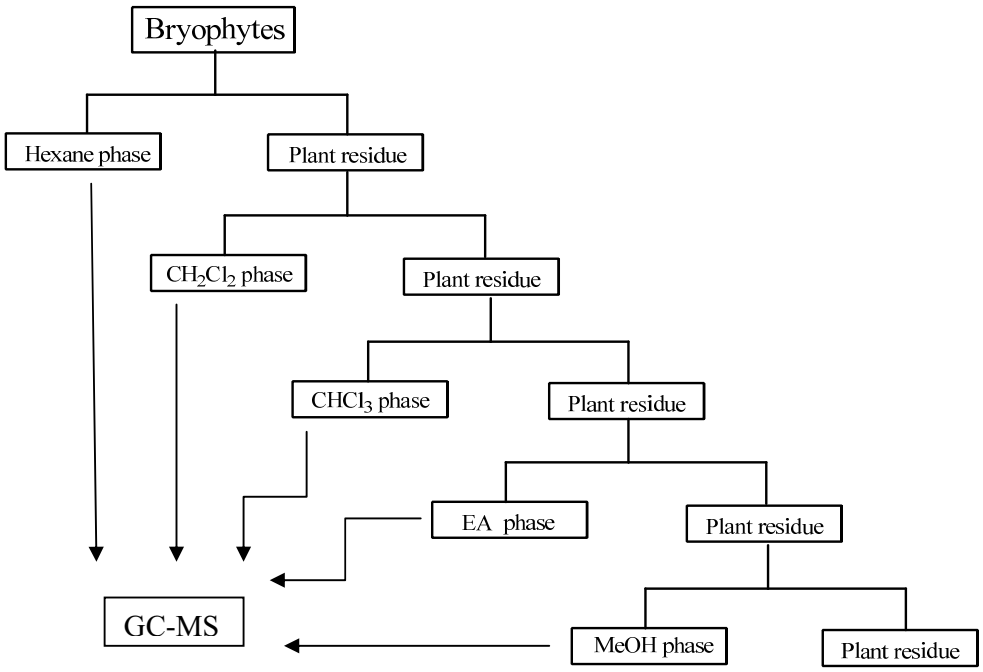

Fig. (1). Scheme of extraction procedures for bryophytes (*Hypnum cupressiforme, Conocephalum conicum, Dicranum scoparium, Homalothecium lutescens* and *Polytrichastrum formosum*).

the mixture was applied at the rate of 1 µl/insect with a 50 µl Hamilton syringe [24]. Controls were determined using acetone. The crushed with air-dried plant extract was applied topically to the dorsal surface of thorax of the insects [25]. The treated insects were transferred to the 60 mm diameter Petri dishes with media. The petri dishes were incubated at 27±2 ºC and in a dark climate chamber, and the number of dead insects was recorded after 24 h. For each replication, 10 insects were used, and each experiment was replicated and repeated three times. A randomized block design was employed including treatments and blank controls.

Statistical Analysis

The results obtained from single-dose screening tests were translated into arcsine values and transformed to percentage mortalities [26]. The resulting values were evaluated by the analysis of variance at the 5% significance level. The data obtained by the analysis of variance were compared using Tukey's multiple comparison test for differences. Statistical analyses were carried out with the help of MINITAB (Release 14) software package [27].

RESULTS AND DISCUSSIONS

Chemical Composition of FAs Contents

The FAs from the Turkish mosses *D. scoparium*, *H. cupressiforme*, *P. formosum*, *H. lutescens* and the Turkish liverwort *C. conicum* were identified in different polarity solvent extracts (hexane, dichloromethane, chloroform, ethyl acetate and methanol, respectively). Each sample (5 g) was powdered with and without liquid nitrogen to see the extraction capacity of the FAs using liquid nitrogen. The FA contents of the liverwort *C. conicum* and the mosses *D. scoparium*, *H. cupressiforme*, *P. formosum*, *H. lutescens* are shown in Tables **1-5** and the main components are shown in Table **6**. Table **1** shows the hexane extracts of various FA methyl esters of *D. scoparium*, *H. cupressiforme*, *P. formosum*, *H. lutescens* and *C. conicum* with the crushing procedures of crushed into small pieces in air-dried conditions' samples (A) and crushed in liquid nitrogen conditions' samples (B). The overall comparison of the two crushing procedures pointed out at hexane as slightly the best solvent to obtain the highest yield rates of saturated fatty acids (SFAs) in crushed with air-dried procedures (A) in all bryophyte samples. However, contents of UFAs, excluding *C. conicum* and *H. lutescens*, were determined as higher in crushed with liquid nitrogen procedure (B). The results indicate different degrees of extraction of SFA and UFAs depending on the crushing procedures in hexane solvent. In the treatment with increasing polarity solvent of the bryophyte samples using dichloromethane, the total content of SFAs were obtained in the highest recovery rates of all bryophytes samples in crushed with air-dried procedure (A), but there was a significant decrease in crushed with liquid nitrogen procedure (B) except for *H. lutescens* (Table **2**). Bryophyte samples extracted with chloroform showed a significant increase in SFA contents in crushed with liquid nitrogen procedure (B). Therefore, in order to evaluate the effect of chloroform extractions on crushed with liquid nitrogen procedure, the concentration of UFAs decreased due to the previous extractions with hexane and chloroform from the same bryophyte samples (Table **3**). The ethyl acetate extraction of the *C. conicum*, *H. lutescens* and *P. formosum* samples gave exclusively SFAs and, in trace content, UFAs in *H. cupressiforme* and *D. scoparium* (Table **4**). We found considerable differences between the methanol and other solvents extraction of all bryophytes using crushed with air-dried procedure (Table **5**). For example, UFA content was found higher in only methanol extracts compared with hexane, dichloromethane, chloroform and ethyl acetate solvents. Although FAs were obtained from all

Table 1. Fatty Acids (%) Obtained with Hexane Extracts of Bryophytes Using Crushed with Air-Dried (A) and Crushed with Liquid Nitrogen (B)

Fatty Acids	*Hypnum cupressiforme*		*Conocephalum conicum*		*Dicranum scoparium*		*Homalothecium lutescens*		*Polytrichastrum formosum*	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
C12:0 Lauric acid								21.32		
C14:0 Myristic acid	4.29				3.51		8.23	9.39	2.29	0.35
C15:0 Pentadecanoic acid					2.17		3.02			
C16:0 Palmitic acid	20.87	16.64	39.57	39.30	24.65	15.31	22.87	30.20	23.48	10.26
C16:0 ethyl ester			4.19						2.61	
C16:1 (7-Z)			3.15							
C16:1 (9-Z)	2.73		20.82	5.62			7.16			0.39
C16:1 (11-E)				4.30		1.23				0.44
C18:0 Stearic acid	9.62		10.66	6.52	14.04	2.80	10.38	23.49	13.80	1.76
C18:1 (7-E)					9.82	11.49	13.60			
C18:1 (9-Z)	19.64	29.70	8.96	9.81		1.48		15.58	14.26	16.94
C18:1 (10-E)										1.18
C18:2 (9,12- cisall)	16.32	33.73	6.80	8.56	7.10	16.86	12.37		14.52	32.42
C18:3 (6,9,12-cis all)						23.84				0.54
C18:3 (9,12,15-cis all)		19.91		13.11	6.19				9.28	24.30
C20:0 Arachidic acid	26.52		5.83	2.92	32.50		22.34		19.73	1.75
C20:1 (11-Z)										
C20:3 (7,10,13-cis all)										1.17
C23:3 (8,11,14-cis all)						2.28				
C20:4 (5,8,11,14-cis all)				5.78		19.20				6.93

solvents and conditions, the methanol extraction by means of crushed with air-dried in *H. lutescens* was not obtained in detection limits, as seen in Table **5**.

These results suggest that the UFA contents decreased with increasing polarity of the solvents in crushed with liquid nitrogen procedures; however, SFAs were in higher contents in crushed with air-dried procedure except methanol extraction. In the literature, some FAs such as 9,12,15-octadecatrien-6-ynoic acid, acetylenic FAs, eicosadien-8-ynoic acid and 9,12-11,14,-octadecadien-6-ynoic acid were reported in many studies on bryophytes [28-31] but were not detected in the present study.

Saturated Fatty Acids in Bryophyte Plants

SFAs in the present work were found varying from capric acid (C10:0) to cerotic acid (C26:0)-with the exception of undecylic acid (C11:0)- in studied bryophyte samples. C10:0 was detected in the ethyl acetate extraction of *D. scoparium* as a little content (0.29). C26:0 was observed in dichloromethane and ethyl acetate extractions of *C. conicum* (5.74) and *P. formosum* (12.36), respectively. However, C26:0 was found in *P. formosum* and *C. conicum* by means of crushed with liquid nitrogen and crushed with air-dried, respectively. Although C21:0 was determined in all studied mosses except in the liverwort of dichloromethane extraction procedure with crushed with air-dried, the chloroform extraction procedure in crushed with liquid nitrogen showed a small amount exclusively in *C. conicum* (0.38). C25:0 was only found in ethyl acetate extraction (3.32) in *P. formosum* crushed in liquid nitrogen. In the present study, we have demonstrated that the bryophytes are a rich source of SFAs (from C12:0 to C24:0) in addition to the FAs mentioned above. The main content of the *C. conicum* and *H. lutescens* in all solvent extractions of crushed with liquid nitrogen and crushed with air-dried is SFAs mainly C16:0 as shown in Table **6**.

Free FA (C16:0-ethyl ester) was determined in hexane, ethyl acetate and methanol extracts of *P. formosum* and hexane and ethyl acetate extracts of *C. conicum* (Tables **1**, **4** and **5**). The same tendency was observed for the first time by Matsuo and coworkers, who reported that the ethyl esters consisting of even-numbered FAs were predominant, and ethyl palmitate (16:0 ethyl ester) was the major constituent [32].

Unsaturated Fatty Acids in Bryophyte Plants

On the basis of plant species in general, proportion of UFAs in *D. scoparium* and *P. formosum* was found to be higher than the other studied species. The UFAs were found from palmitoleic and palmitelaidic acids (16:1 cis-9 and trans-9, respectively) to nervonic acid (24:1, cis-15) in studied bryophyte samples. Interesting results were observed

Table 2. Fatty Acids (%) Obtained with Dichloromethane Extracts of Bryophytes Using Crushed with Air-Dried (A) and Crushed with Liquid Nitrogen (B)

Fatty Acids	*Hypnum cupressiforme*		*Conocephalum conicum*		*Dicranum scoparium*		*Homalothecium lutescens*		*Polytrichastrum formosum*	
	A	B	A	B	A	B	A	B	A	B
C12:0 Lauric acid	3.94	0.92	0.42		2.07		31.84	14.35	0.20	
C14:0 Myristic acid	2.43	1.25	0.46	0.58	1.49		5.32	3.67	1.57	
C15:0 Pentadecaonic acid	0.36	0.60		0.37	0.48		0.46		0.17	
C16:0 Palmitic acid	7.93	13.38	17.86	31.54	12.55	2.75	0.51	15.32	12.78	11.89
C16:1 (9-E)				1.90						
C16:1 (9-Z)		0.79	0.52	13.37			0.54	1.56		
C17:0 Margaric acid							0.88		0.24	
C18:0 Stearic acid	2.46	2.37	2.44	3.05	4.17	0.43	2.50	3.35	7.00	1.32
C18:1 (9-E)				14.28						
C18:1 (9-Z)	1.01	13.52		3.84	2.55	1.63	2.47	9.96	0.77	15.25
C18:2 (9,12-all cis)		20.58		6.73	0.91	2.85	0.44	9.72	0.16	32.30
C18:3 (9,12,15-cis all)		13.38		7.28		5.14		7.19		22.10
C20:0 Arachidic acid	9.33		2.46	0.89	14.07	0.68	5.58	4.94	16.41	2.48
C20:1 (11-Z)				0.35						0.92
C20:3 (7,10,13-cis all)				0.54		0.50				
C20:4 (5,8,11,14-cis all)		6.53		2.87		4.17		8.75		5.29
C21:0 Heneicosanoic acid	0.42				0.57		0.31		0.74	
C 22:0 Behenic acid	37.43	12.86	23.37	6.56	27.78	1.77	17.19	21.20	40.98	3.85
C22:6 (4,7,10,13,16,19 cis all)					5.65	80.57				
C 24:0 Tetracosanoic acid	33.69	10.32	41.27	5.00	26.25	1.02	22.06	13.83	17.50	2.16
C 24:1 (15-Z)										1.45
C26:0 Cerotic acid			5.74							

on FA composition of the hexane extraction using crushed with liquid nitrogen and crushed with air-dried procedures. We found a high UFA (alpha-linolenic acid, C18:3) level in the crushed with liquid nitrogen extraction procedure and a low SFA level (arachidic acid, C20:0) in the crushed with air-dried procedure with hexane extraction (Fig. **2**). Docosahexaenoic acid (C22:6) was exclusively found in *D. scoparium* (80.57) extracted with dichloromethane, as seen in Table **2** and Fig. (**3**). The distribution of PUFAs (C22:6) in bryophytes of the present work has chemotaxonomic relevance using solvents extractions from nonpolar (hexane) to polar (methanol). Dembitsky and Rezanka stated that certain FAs, for example, acetylenic and some polyunsaturated acids are obvious markers [33]. The main UFA of *H. cupressiforme* (33.73) (hexane) and the second in the dichloromethane extraction of *P. formosum* (32.30) was linoleic acid incrushed with liquid nitrogen (Tables **1** and **2**).

The elaidic acid was the main constituent of UFA in chloroform extraction of *P. formosum* (11.79), *H. cupressiforme* (11.49), *D. scoparium* (9.77) (Table **3**), and in ethyl acetate extraction of *D. scoparium* (3.51) (Table **4**). However, the main UFA of methanol extract was alpha-linolenic acid (36.46) (Table **5**). Treatment with increasing solvent polarity and crushing process of bryophyte samples exhibited significant changes in the SFA and UFA levels between the crushed with liquid nitrogen and the crushed with air-dried methods. Therefore, UFA rates were significantly decreased in crushed with liquid nitrogen procedure in protic polar solvent (methanol), yet SFA content was remarkably increased (Fig. **4**). However, no significant changes in the ratio of cis and trans FAs were found (Fig. **4**). All investigated mosses,16:1, 18:1 and 18:2 UFAs were found in all samples regarding the 20 UFAs. It is evident that with no exception, C22:6 is the dominant UFA extracted with dichloromethane from *D. scoparium* in crushed with liquid nitrogen (80.57) and crushed with air-dried (5.65) (Tables **2** and Fig. **3**).

Insecticidal Activity Bioassay

Methanol extract of *H. cupressiforme* was treated with diluted acetone (50 µg/µl) to give the highest mortality rate (51.13 %) and was statistically different from other extracts (F=163,32; d.f.: 5,12; P=0,000). The other tested plant extracts showed 12,18-37,77% mortality for 24h (Table **7**). As a result of the studies carried out with hexane extracts, insecticidal activities of bryophyte extracts increased and the extracts showed mortality between 37,69-70,33 % (F=96,92; d.f.: 5,12; P=0,000). Although the highest activity in hexane

Table 3. Fatty Acids (%) Obtained with Chloroform Extracts of Bryophytes Using Crushed with Air-Dried (A) and Crushed with Liquid Nitrogen (B)

Fatty Acids	*Hypnum cupressiforme*		*Conocephalum conicum*		*Dicranum scoparium*		*Homalothecium lutescens*		*Polytrichastrum formosum*	
	A	B	A	B	A	B	A	B	A	B
C12:0-10-methyl									1.07	
C12:0 Lauric acid	3.12	1.93	0.78	0.19	0.86	0.15	8.20	6.13		0.33
C13:0 Tridecylic acid				0.03		0.07	0.21			
C14:0 Myristic acid	2.18	2.90	0.85	1.75	1.80	1.37	3.00	3.27	2.25	1.60
C14:0-12-methyl		0.62						0.66		0.10
C15:0 Pentadecanoic acid	0.97	1.84	0.44	1.00	1.42	3.02	1.17	1.24	1.10	0.87
C15:0-14-methyl		0.73			0.46		0.62	0.51		
C15:0-12-methyl					0.65	2.18	0.52	0.32		
C16:0 Palmitic acid	13.34	43.65	31.51	46.06	32.28	55.84	17.80	36.67	33.41	40.39
C16:1 tr-9			6.70		0.62	1.44	0.27	0.39	1.92	
C16:1 cis-9						0.60		0.19		
C16:0-14-methyl		0.57			0.77	0.80		0.34		
C16:0-3,7,11,15-tetramethyl					0.86		7.66	3.23		1.13
C17:0 Margaric acid		1.09	0.34	1.30	1.30	2.50	0.73	0.75		0.96
C17:0-14-methyl						0.96				
C17:1(cis-10)						0.12	0.58			
C18:0 Stearic acid	6.99	5.66	3.12	7.13	8.66	3.78	3.37	3.80	7.85	5.52
C18:1 tr-9	11.49		4.08	0.87	9.77	2.77	9.18	2.78	11.79	
C18:1 cis-9			2.89		1.78	1.90	2.27			
C18:2 (9,12-all cis)					3.89	6.56	8.27	2.12		
C18:3 (9,12,15-cis all)					2.40	7.28	2.91	3.51		
C19:0 Nonadecylic acid				0.54						
C20:0 Arachidic acid	6.86	6.42	2.33	11.12	7.42	0.98	5.16	3.87	9.47	25.56
C20:3 (cis-8,11,14)						0.64				
C20:4 (cis-5,8,11,14)						2.93	2.33			
C21:0 Heneicosanoic acid				0.38						
C22:0 Behenic acid	26.88	18.05	16.60	15.51	12.84	1.26	14.43	17.08	19.45	14.10
C23:0 Tricosanoic acid			4.21	0.97						
C24:0 Tetracosanoic acid	28.17	16.53	26.15	13.17	12.24	2.85	11.31	13.14	11.71	9.44

extracts of *P. formosum* (70.33%) was seen, *D. scoparium* (58.92%) and *H. lutescens* (55.57%) were statistically found in the same group. The lowest activity among the extracts was in *C. conicum* (37.69%) (Table **7**). The studies were carried out with esterified methanol extracts, and the highest activity was obtained from *D. scoparium* (43.32%). *H. cupressiforme* (37.75%) and *H. lutescens* (36.64%) statistically took place in the same group with *H. lutescens* (36.64%). The activities of other extracts were observed as low (F=132.44; d.f.: 5,12; P=0,000), (Table **7**).

Contact toxicity activities were studied with lauric, myristic and palmitic acids besides single dose studies of the solvent extracts. The highest mortality rate (53.34%) was obtained from the myristic acid among the tested pure FAs. The activities of palmitic and lauric acids were 17.75% and 4.32%, respectively (F=172.55; d.f.: 3,8; P=0,000), (Table **7**). Zhang, *et al*. [34] reported that the proper length of the side chain of the components was very important for their insecticidal activity. Therefore, the insecticidal activity results of the methanol, hexane esterified methanol extracts and pure FAs in the present study were compatible with the literature.

The insecticidal activity of hexane extracts showed the highest mortality in *P. formosum*. However, the rate of myristic acid (C14:0) is only 1.57% in whole extracts. The comparison with the pure myristic acid's insecticidal activity showed that the higher activity in *P. formosum* was not only coming from the myristic acid in hexane extract of *P.*

Table 4. Fatty Acids (%) Obtained with Ethyl Acetate Extracts of Bryophytes Using Crushed with Air-Dried (A) and Crushed with Liquid Nitrogen (B)

Fatty Acids	*Hypnum cupressiforme*		*Conocephalum conicum*		*Dicranum scoparium*		*Homalothecium lutescens*		*Polytrichastrum formosum*	
	A	B	A	B	A	B	A	B	A	B
C10:0 Capric acid						0.29				
C12:0-10-methyl	0.17									
C12:0 Lauric acid	1.22		1.58		1.40	0.95	16.55	3.06		
C13:0 Tridecylic acid						0.23				
C14:0 Myristic acid	4.74	4.80	2.93	1.26	3.55	4.61	7.69	5.24	5.33	0.89
C14:0-12-methyl	1.54	1.56	1.10		1.35	2.31		0.49		
C15:0 Pentadecanoic acid	2.49	2.53	2.41	2.21	2.82	3.75	2.95	1.92	3.20	0.94
C15:0-14-methyl					0.95					
C16:0 Palmitic acid	35.41	36.05	67.89	59.20	46.64	53.55	42.15	50.88	78.33	48.56
C16:0-ethyl ester			2.45						2.13	
C16:1 tr-9	1.05	1.06			1.25	2.74				
C16:0-14-methyl	1.51	1.54			2.37	1.18	1.39			
C16:0-3,7,11,15-tetramethyl				4.30	2.45		10.29	15.12		
C17:0 Margaric acid	1.32	1.34			2.22	2.57		0.70		0.98
C18:0 Stearic acid	9.87	10.04	6.70	7.99	8.85	8.37	7.26	5.08	11.01	5.09
C18:1 tr-9						3.51				
C18:2 cis-9,12						1.75				
C20:0 Arachidic acid	8.42	8.58			5.67	2.99	3.28	2.10		4.83
C22:0 Behenic acid	18.32	18.64	14.94	10.59	12.77	6.16	8.44	6.38		4.00
C23:0 Tricosanoic acid							10.00			0.81
C24:0 Tetracosanoic acid	13.94	14.19		14.45	7.70	5.05		9.02		18.21
C25:0 Pentacosylic acid										3.32
C26:0 Cerotic acid										12.36

formosum. The higher activity might be due to the synergic effect of the other FAs. Methanol extract of *P. formosum* exhibited the lowest activity (12.18% in Table **7**) in all tested samples can be comparable with the similar rate of palmitic acid (20.33% for methanol extract, 23.48% for hexane extract; ratio of 1.00:1.15) and different rate of myristic acid (0.25% for methanol extract, 2.29% for hexane extract; ratio of 1.00:9.16) could be the case for the different activity of hexane and methanol extracts.

CONCLUSIONS

In this study, the FA extractions and insecticidal activities of bryophytes were evaluated using different organic solvents. The FA samples were prepared using crushed with liquid nitrogen and crushed with air-dried methods. The ratio of SFA and UFAs are different in terms of crushed with liquid nitrogen and crushed with air-dried conditions. The main component was generally palmitic acid (C16:0) in all bryophytes, except *D. scoparium* (Table **6**). As the solvent extracts of hexane and methylene chloride phases were analyzed, the SFAs ratio was found to be higher than in chloroform, ethyl acetate and methanol. In addition, methyl-branched FAs have been identified in hexane and methylene chloride extracts but has not been detected in chloroform and methanol extracts. Regarding the effect of the crushing process with liquid nitrogen, hexane extracts increased the rate of UFAs, whereas when methanol extract is reduced, a significant change was not observed in chloroform and ethyl acetate extracts. All results were evaluated and the highest impact was observed at hexane extracts. The acting substances in hexane and methanol extracts were the same. But the ratio of these substances was high and this suggested a high activity.

Insecticidal activity studies show that the highest effect was obtained from the hexane extract of *P. formosum*. The second highest activity was gained from the hexane extract of *D. scoparium* and the following activities were hexane extracts of *H. lutescens* and *H. cupressiforme*. The lowest activity was acquired from the methanol extract of *P. formosum*. The highest activities among the studied solvent extracts were achieved from the hexane extract. The insecticidal activity decreased with esterification of FAs. The evaluation of insecticidal activity of individual FAs (lauric,

Table 5. Fatty Acids (%) Obtained with Methanol Extracts of Bryophytes Using Crushed with Air-Dried (A) and Crushed with Liquid Nitrogen (B)

Fatty Acids	*Hypnum cupressiforme*		*Conocephalum conicum*		*Dicranum scoparium*		*Homalothecium lutescens*		*Polytrichastrum formosum*	
	A	B	A	B	A	B	A	B	A	B
C12:0-10-methyl				0.05						
C12:0 Lauric acid	0.17	0.19	0.10	0.09	0.04	0.15		0.27		0.06
C13:0 Tridecylic acid	0.05	0.05	0.07			0.07				
C13:0-12-methyl	0.33		0.27	0.43	0.18	0.44				
C14:0 Myristic acid	1.36	1.85	0.64	1.00	0.53	1.37		1.67	0.25	0.61
C14:0-12-methyl	1.74	1.92	1.66	2.94	0.57	1.74		0.90	0.14	0.05
C15:0 Pentadecanoic acid	0.83	2.73	0.73	1.61	0.95	3.03		1.29	0.27	0.87
C15:0-14-methyl	0.59	1.40	0.82	1.64	0.28	0.07		0.84		0.05
C15:0-12-methyl	0.05			0.10		0.80				
C16:0 Palmitic acid	19.96	73.34	28.71	68.00	25.07	56.03		56.56	20.33	40.61
C16:0-ethyl ester										0.55
C16:1 (tr-7)	0.59		0.86		0.67	0.57			0.41	0.26
C16:1 (tr-9)	3.67	0.09	3.07	0.35	1.34	1.44		1.93	0.45	0.05
C16:1 (cis-9)	1.50	0.40	3.58	0.39	0.60	0.60		1.08		
C16:0-14-methyl	0.81	0.95	0.55	1.04				0.16		
C16:0-15-methyl	0.36							0.36		
C16:1-(11-cis) 15-methyl	0.19		0.48	1.33						
C16:0-3,7,11,15-tetramethyl			0.51	0.60				5.17	0.45	
C16:2 (tr-7,10)			1.27						1.47	0.69
C16:3 (tr-7,10,13)	1.35		4.74							
C17:0 Margaric acid	0.45	1.34	0.51	1.30	1.06	2.50		0.58	0.52	1.63
C17:0 10-methyl		0.94		0.33						
C17:1(cis-9)										0.28
C17:1(cis-10)					0.64	0.12				0.11
C18:0 Stearic acid	1.89	2.84	1.90	4.43	1.77	3.80		2.21	1.41	3.56
C18:1 (tr-9)	9.98	2.80	6.09	2.14	4.42	2.78		4.63	8.65	9.82
C18:1 (cis-9)	5.84	1.17	5.14	1.44	3.43	1.91		2.94	1.23	1.22
C18:2 (cis-9,12)	19.75	2.83	8.83	1.21	16.62	6.59		7.60	28.11	17.14
C18:2 (tr-10,13)					0.18					0.08
C18:3 (cis-6,9,12)	0.57		0.37		3.25				26.89	0.25
C18:3 (cis-9,12,15)	15.73		22.39	1.16	36.46	7.31		7.94		9.31
C19:0 Nonadecylic acid				0.21						0.13
C19:1 (tr-10)		0.97								
C20:0 Arachidic acid	0.71	0.78	0.43	0.98	0.64	0.98				0.73
C20:1 (cis-11)			0.31							0.72
C20:2 (cis-11,14)	0.58		0.23							0.50
C20:3 (cis-8,11,14)	0.58		0.42	2.70		0.64				0.72
C20:4 (cis-5,8,11,14)	5.44		3.32	4.55		2.94		3.87	9.31	0.29
C22:0 Behenic acid	3.36	1.38				1.27				0.74
C23:0 Tricosanoic acid			0.36							0.48
C24:0 Tetracosanoic acid	1.57	2.05	1.66		1.31	2.86				7.04
C24:1 (cis-15)										1.45

Table 6. The Main Components (%) of the Bryophytes' Fatty Acids Extracted with Five Solvents from Nonpolar to Polar

Solvents	*Hypnum cupressiforme*		*Conocephalum conicum*		*Dicranum scoparium*		*Homalothecium lutescens*		*Polytrichastrum formosum*	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
Hexane	26.52 (C20:0)	33.73 (C18:2)	39.57 (C16:0)	39.30 (C16:0)	32.50 (C20:0)	23.84 (C18:3)	22.87 (C16:0)	30.20 (C16:0)	23.48 (C16:0)	32.42 (C18:2)
CH_2Cl_2	37.43 (C22:0)	20.58 (C18:2)	41.27 (C24:0)	31.54 (C16:0)	27.78 (C22:0)	80.57 (C22:6)	31.84 (C12:0)	21.20 (C22:0)	40.98 (C22:0)	32.30 (C18:2)
$CHCl_3$	28.17 (C24:0)	43.65 (C16:0)	31.51 (C16:0)	46.06 (C16:0)	32.28 (C16:0)	55.84 (C16:0)	17.80 (C16:0)	36.67 (C16:0)	33.41 (C16:0)	40.39 (C16:0)
Ethyl Acetate	35.41 (C16:0)	58.76 (C16:0)	67.89 (C16:0)	59.20 (C16:0)	46.64 (C16:0)	53.55 (C16:0)	42.15 (C16:0)	50.88 (C16:0)	78.33 (C16:0)	48.56 (C16:0)
Methanol	19.96 (C16:0)	73.34 (C16:0)	28.71 (C16:0)	68.00 (C16:0)	36.46 (C18:3)	56.03 (C16:0)	-	56.56 (C16:0)	28.11 (C18:2)	40.61 (C16:0)

A crushed with air-dried, B crushed with liquid nitrogen.

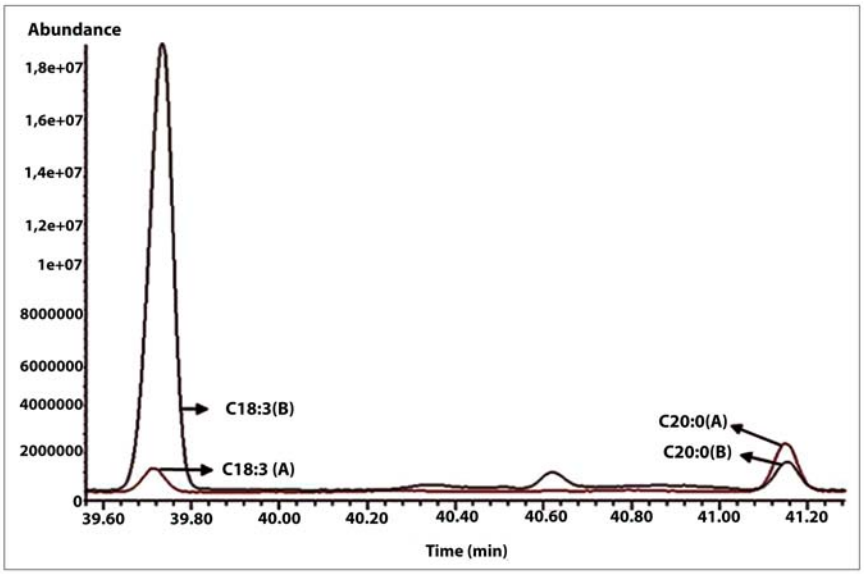

Fig. (2). Fatty acid methyl ester chromatogram of *Polytrichastrum formosum* for alpha-linolenic acid (18:3) and arachidic acid (20:0) extracted with hexane using crushed with air-dried (A) and crushed in liquid nitrogen (B) methods.

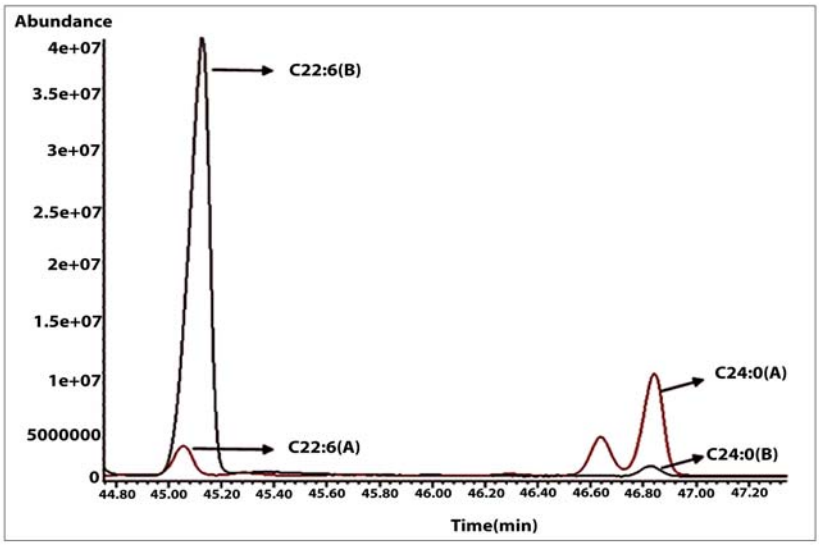

Fig. (3). Fatty acid methyl ester chromatogram of *Dicranum scoparium* for lignoceric acid (24:0) and docosahexaenoic acid (22:6) extracted with dichloromethane using crushed with air-dried (A) and crushed in liquid nitrogen (B) methods.

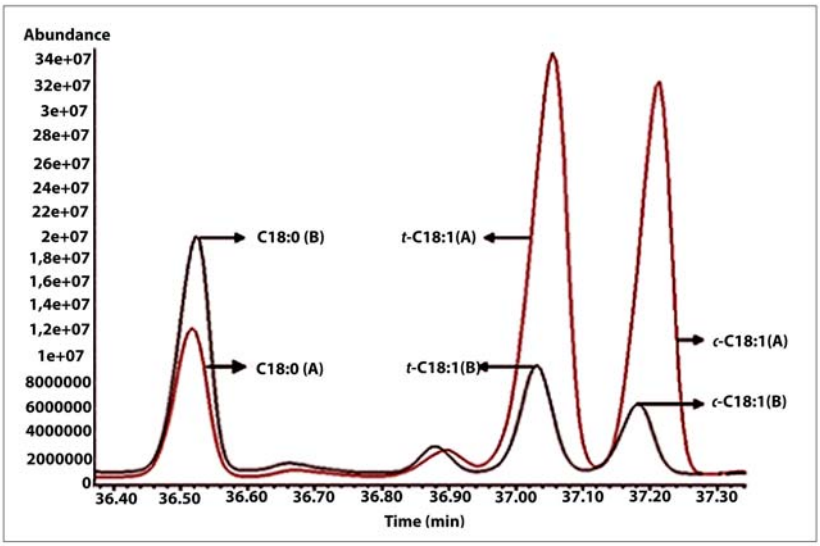

Fig. (4). Fatty acid methyl ester chromatogram of *Conocephalum conicum* for stearic acid (18:0), elaidic acid (18:1, 9E) and oleic acid (18:1, 9Z) extracted with methanol using crushed with air-dried (A) and crushed in liquid nitrogen (B) methods.

Table 7. Insecticidal Effect of Different Bryophyte Extracts against *S. granarius* for 24 h

Experiment	% Mortality±SE			
	Methanol Extracts	Hexane Extracts	Esterified Methanol Extracts	Fatty Acids
Control	0,00±0,00d[1]	0,00±0,00d	0,00±0,00d	0,00±0,00
Hypnum cupressiforme	51,13±0,71aAB	53,34±0,11bcA	37,75±0,16aB	--
Conocephalum conicum	31,10±0,04bA	37,69±0,52cA	13,21±0,24cB	--
Dicranum scoparium	37,77±0,04bB[2]	58,92±0,26abA	43,32±0,11aB	--
Homalothecium lutescens	37,77±0,04bB	55,57±0,27abA	36,64±0,12aB	--
Polytrichastrum formosum	12,18±0,09cB	70,33±1,22aA	24,33±0,35bB	--
C12:0; Lauric acid	--	--	--	4,32±0,20
C14:0; Myristic acid	--	--	--	53,34±0,11
C16:0; Palmitic acid	--	--	--	17,75±0,06

[1]Means in a column followed by a different lower case letter are significantly different (Anova $P < 0.05$, Tukey test).
[2]Means in a line followed by a different upper case letter are significantly different (Anova $P < 0.05$, Tukey test).

myristic and palmitic acids) and the existence of possible synergism among solvent extracts of FAs, with the employment of sophisticated assays, stands as a challenging perspective in order to further pinpoint insecticidal activity of FAs.

The ecofriendly, pollution-free and easily degradable bio-pesticides could be thought to be an alternative against synthetic components for insects. The biologically active components produced by plants are typically primer and seconder metabolites such as alkaloids and lipids. These defensive metabolites are bio-compatible with non-residual effects on the ecosystem. Therefore, FA extracts obtained from bryophytes may be suitable against stored product pests.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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