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Total amount of protein, lipid and carbohydrate of some adult species belong to curculionidae family (Coleoptera: Curculionidae)

Hakan Bozdoğan, Mahmut Erbey and Hatice Avan Aksoy

Abstract
Laboratory studies were conducted to determine total protein, lipid and carbohydrate concentrations of adults 15 species belong to 12 genera (Coleoptera: Curculionidae). Due to the structural elements of the protein, protein amount has been founded high in all species. On the other hand carbohydrate has been founded low in all treated species. These results indicated that there is no relationship between the wet weight and the amount protein, lipid and carbohydrate. Also, protein, lipid and carbohydrate levels showed some variations even in the same genera.

Keywords: Coleoptera, curculionidae, lipid, protein, carbohydrate

1. Introduction
Curculionidae is considered to be one of the most richest families in Coleoptera in terms of total number of species. To date, the existence of 60.000 species belonging to the family Curculionidae was determineted in the world and 13.000 species were described from Palearctic region. Also, the family Curculionidae, covering the study samples, is the organism groups having economic importance. Namely, all of species in the family Curculionidae are phytophag with exception of several species (Ross, 1963) [10]. Larval and adult stages feed on plant organs like; roots, stems, leaves and fruit, and can have a detrimental effect on crops and forest trees causing economic losses (Mihajlova, 1978) [6]. Family species spend the winter usually mature form. With the introduction of insect activity in the spring, adults begin to do damage. Female insects on host plants open holes with rostrum. Into these holes, one generally lays eggs. Hatched larvae begin feeding location. The period is the most damaging larval stage. After spending pupa period on the same plant or in the soil, the adult continues to damage (Richard and Davies, 1977) [11]. The accurate determination of the taxonomic positions of species by using biochemical agents in the family will directly contribute pest control efforts for these organisms leading to plant damage and economic losses. Therefore, knowing their biochemical structure is of paramount importance in the process of choosing biological control agents.
However, until now, very little has been done in terms of describing and understanding the protein, lipid and glucose profile of Curculionidae (Shapiro, 1988; Due *et al.,* 2009) [14, 3]. These studies also are restricted with larvae.
The objective of the present study is to evaluate the protein, lipid and carbohydrate levels of adult species belong to Curculionidae. The data may be helpful in formulating some strategies for biological control.

2. Materıals And Methods
2.1 Insects Homogenization for the determination of protein content
For protein analysis, insect species wet weights received and freeze stocked received to assay tubes. After standed at room temperature thawed and several fenilthiour crystalline added to prevent melaninleş and homogenized with a homogenizer 24000 rev/min by adding 1/5 phosphate buffer (pH 7.4). After homogenization, tubes centrifuged at 6000 rev/min for 30 min. Determination of protein was carried out by taking 1 ml. supernatant content from tubes.

2.2 Determination of Protein Content

Solutions

Solution A: [2%Na_2CO_3 (0.1 N NaOH)] 2 g of Na_2CO_3 were weighed and 0.1 N NaOH dissolved. Total volume 100ml.

Solution B1: (1% $CuSO_4.5H_2O_2$): 1 g $CuSO_4.5H_2O$ was weighed and solution solved with bidistilled water. Total volume 100 ml.

Solution B2: (2% Na-K-tartrate) 2 g of Na-K-tartrate solution was weighed and solved in the pure water.

Solution C: 50 volume solution A was stirred with 1 volume, 1/1 ratio B1 and B2 a mixture.

Folin-Ciocalteu solution: Before using diluted ratio of 1/1.5 with bidistilled pure water.

Before measuring Protein content 100 ml a stock solution cantaining albumin (1g/100ml) was prepared and standard solutions (0.10, 0.50, 1.00, 2.00, 4.00, 6.00, 8.00 ve 10.00 mg/ml) were obtained from this solution by diluting. The light absorption was read at 750 nm and standard protein graph (regression line) was obtained from dataes.

Protein assay method was applied to each standard solution (Lowry *et al*., 1951). in a spectrophotometer at 750 nm was read and the data values

$y = 1.242x + 0,570$ ($R^2 = 0,981$)

Protein quantities of samples, was calculated by using this regression equation.

To determination of the protein content, 0.3 ml bidistilled pure water was added to blind tube and 3 ml of solution C was added on. After standing at the room temperature for 15minutes, 0.3 ml of Folin-Ciocalteu was added. After standing for 30 minutes, values were readed at 750 nm. For reading samples, 0.3 ml of solution C was added onto 0.3 ml sample.and than standed for 15 min at room temperature. Than it was incubated for 30 min. by additioning of 0.3 ml Folin-Ciocalteu. Then the absorbance values were read against blank at the 750 nm. The obtained light absorption value substituted into the regression line equation. Thus, insects' total protein content was obtained in a repetition of a series of experiments.

Per individual protein content were detected by dividing this value to the number of insect. Protein rate per individual according to wet weight was obtained by multipliying by 100 and dividing to wet weight of protein content per individual.

2.3 Insects Homogenization for Determinationing of Lipid Content

For lipid analysis, the stocked insects received wet weights and freezed were taken to assay tubes and standed at the room temperature. After thawing, several drops fenilthiour crystalline added to prevent of melanisation. Also 2 ml (2%) sodium sulfate was added homogenized with a homogenizer 24000 rev/min. After homogenization process, 5 ml of chloroform / methanol (1/2) solutions was added into tubes. And centrifuged 10 min 6000 rev /min. 1 ml supernatant was used for lipid analysis.

2.4 Determination of Lipid Content

Solutions

1. 2% Na_2SO_4: 2 g of Na_2SO_4 was weighed in the flask joje and was filled up to 100 ml with distilled water.
2. Vanillin-phosphoric acid: 600 mg vanillin were dissolved in 100 ml hot water and stirred with 400 ml of 85% phosphoric acid and kept in the dark.
3. Concentrated Sulfuric Acid (%95–97) (H_2SO_4)
4. Chloroform / methanol mixture (1/2): 10 ml of chloroform and 20 ml methanol was stirred in flask which was tightly sealed and stored.

For analysis, determining the amount of total lipid in the samples stored, Van Handel (1985b) [20] method were used.

To determine of lipid values, firstly standard lipid graphic was drawn. For this 0.1% olive oil was used. Stock standard solution concentration was accorded as 1 mg/ml by using chloroform / methanol (1/2) solutions. Then, 0.10, 0.50, 1.00, 2.00, 3.00, 3.50, 4.00, 5.00, 7.50 and to 10.00 mg / ml solutions were prepared by dilutioning this solution. 200 µl of this solution was transferred into test tubes. These tubes was heated in a water bath at 90 °C until chloroform / methanol solution vaporise completely. 40 µl concentrated sulfuric acid solution was added onto tubes were taken from water batch. the tubes were mixed by vortex and heated in the bath at 90 °C for 2 minutes. Then 960 µl reactive vanillin-phosphoric acid prepared by the method of Van Handel (1985b) [20] was added into cooled each tubes. The tubes mixed for 30 minutes at room temperature and a color formation was provided. Finally the tubes were mixed and the absorbance values of the tubes was read at a wavelength of 525 nm in a spectrophotometer against to blind. These processes were repeated three times for each standard solution concentrations. Standard lipid graph (regression curve) was drawnby using absorbance values.

$y = 0.325x + 1.111$ ($R^2 = 0.814$)

Examples of the lipid content were calculated by the regression equation.

For lipid analysis, 1 ml sample were taken from supernatant formed at the end of the centrifuge were taken and transferred to the testing tubes. These tubes containing chloroform / methanol solution were heated in a water bath at 90 °C until complete evaporation.

Precipitate remaining in the tubes through the lipid, by the addition of 2 ml of concentrated sulfuric acid solution and the tubes were mixed with vortex for 2 minutes in a water bath heated at 90 °C. Then cooled on each tube, 5 ml vanillin-phosphoric acid reagent was added, the tubes were left at room temperature for 30 min and color formation was provided. Finally the tubes were mixed and the absorbance values of the tubes in a spectrophotometer at a wavelength of 525 nm was read against blank. Readed absorbance values were substituted in the standard graph. And total lipid content were determined.

2.5 Insect Homogenization for Determination of carbohydrates content

For Carbohydrate analysis. the insects wet weights received and freeze stocked are received to assay tubes and standed at room temperature. After thawed several drops fenilthiour crystalline added to prevent melaninleş. And added 2 ml (2%) sodium sulfate onto samples. Than it was homogenized with a homogenizer 24000 rev/min. After homogenization 5 ml of chloroform/methanol (1/2) solution was added into tubes and centrifuged at 10 min a of 6000 rev/min. 1 ml supernatant in the tubes was taken to analyze the carbohydrate analisis.

2.6 Determination of carbohydrate content

In the determination of carbohydrate content Van Handel (1985) [19, 20] method was used.

Solutions;

1. 1.Antron Solution: 750 mg antron were dissolved into dissolved in 150 ml bidistilled pure water and 380 ml concentrated H_2SO_4
2. 2% sodium sulfate (Na_2SO_4) Solution: 2 g of Na_2SO_4 was weighed and solved in the bidistilled water.
3. Chloroform/Methanol Mixture (1/2): 10 ml of chloroform and 20 ml methanol was stirred in the flask mayer, tightly sealed and stored

Before the determination amount of Carbohydrate stock solution was prepared containing0.1 g of pure glycogen (Sigma G-8751) in a 1 ml. and 0.10, 0.50, 1.00, 2.50, 3.00, 4.00, 5.00, 7.50, 10.00 mg / ml glycogen standard solutions were obtained by dilutioning. This Van Handel glycogen standard series (1985a) [19] method is applied to samples. Samples were read at 625 nm wavelength in a spectrophotometer and standard graph of glycogen (regression line) was drawn for obtained dataes.

$y = 0.393x + 0.504$ ($R^2 = 0.906$)

For carbohydrate analysis, 1 ml samples were taken from supernatant the formed at the end of the centrifuge and transferred to the testing tubes. These tubes containing chloroform / methanol solution were heated in a water bath at 90 °C until complete evaporation. After cooling tubes, 5 ml antron solution were added and than incubated at 15 min. at 90 °C. Tubes's absorbance were readed at 625 nm in a spectrophotometer. Readed absorbance values substituted into the regression equation. Amount of carbohydrate in the samples (1ml) was obtained terms of mg.

3. Results

Table 1: Total protein, carbohydrate and lipid values per individual in all species

No	Species Name	Average per individual Wet weight	Protein Amount (mg/ml)	Carbohydrate Amount (mg/ml)	Lipid Amount (mg/ml)
1	*Phyllobius karamanensis*	8 mg	1,718±0,3	0,279±0,03	0,636±0,25
2	*Oedecnemidius saltuarius*	6 mg	0,518±0,01	0,223±0,02	0,502±0,08
3	*Polydrusus spp.*	3 mg	0,998±0,04	0,455±0,12	0,100±0,01
4	*Curculio nucum*	15 mg	2,226±0,15	0,367±0,07	0,860±0,17
5	*Curculio glandium*	17.5 mg	10,563±0,23	1,611±0,15	3,527±0,53
6	*Curculio villosus*	5 mg	0,600±0,05	0,107±0,05	0,235±0,17
7	*Hypera postica*	10 mg	4,030±0,13	0,615±0,18	1,399±0,19
8	*Gymnaetron asellus*	12.5 mg	1,777±0,10	0,255±0,21	0,646±0,08
9	*Gymnaetron tetrunn*	4 mg	1,435±0,09	0,224±0,03	0,503±0,04
10	*Zacladus asperactus*	3.75 mg	5,357±0,08	0,927±0,04	1,945±0,54
11	*Lixus cardui*	41.4 mg	8,030±0,14	1,353±0,74	3,257±0,71
12	*Larinnus latus*	286 mg	1,632±0,19	0,245±0,05	0,475±0,06
13	*Baris spp.*	5 mg	0,675±0,06	0,143±0,03	0,244±0,04
14	*Sitona hunneralis*	5 mg	1,079±0,11	0,176±0,05	0,383±0,08
15	*Ceutorhynchus spp.*	2 mg	0,107±0,01	0,113±0,14	0,257±0,09

Table 2: Per individual total and percentage of carbohydrate amount

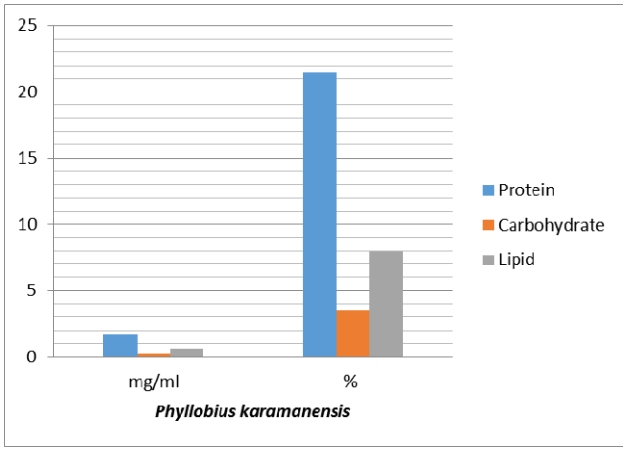
No	Species Name	Average per individual wet weight	Carbohydrate amount (mg/ml)	Percentage of carbohydrate amount(%)
1	*Phyllobius karamanensis*	8 mg	0,279±0,03	3,487±0,17
2	*Oedecnemidius saltuarius*	6 mg	0,223±0,02	3,716±0,11
3	*Polydrusus spp.*	3 mg	0,455±0,12	15,166±1,27
4	*Curculio nucum*	15 mg	0,367±0,07	2,446±0,87
5	*Curculio glandium*	17.5 mg	1,611±0,15	9,205±0,63
6	*Curculio villosus*	5 mg	0,107±0,05	2,14±0,14
7	*Hypera postica*	10 mg	0,615±0,18	6,15±0,98
8	*Gymnaetron asellus*	12.5 mg	0,255±0,21	2,04±0,41
9	*Gymnaetron tetrunn*	4 mg	0,224±0,03	5,6±0,97
10	*Zacladus asperactus*	3.75 mg	0,927±0,04	24,72±1,15
11	*Lixus cardui*	41.4 mg	1,353±0,74	3,268±0,75
12	*Larinnus latus*	286 mg	0,245±0,05	0,085±0,04
13	*Baris spp.*	5 mg	0,143±0,03	2,86±0,73
14	*Sitona hunneralis*	5 mg	0,176±0,05	3,52±0,54
15	*Ceutorhynchus spp.*	2 mg	0,113±0,14	5,65±0,68

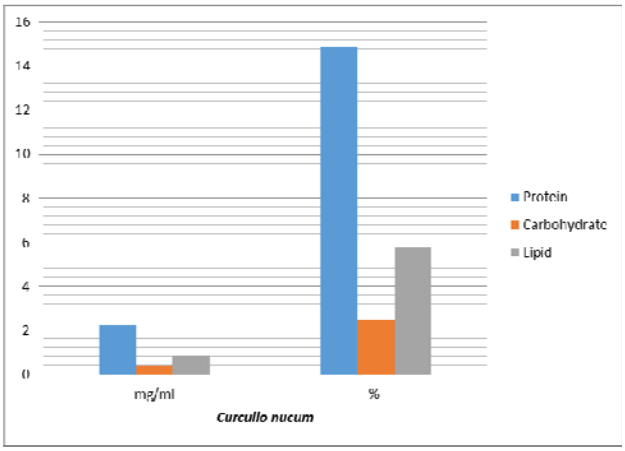
Table 3: Per individual total and percentage of lipid amount

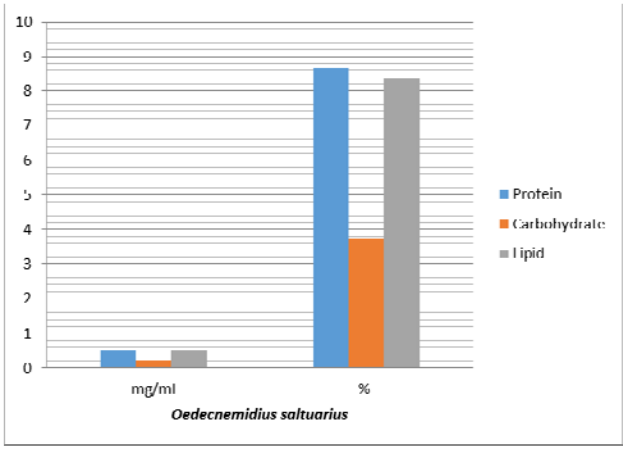
No	Species name	Per individual average Wet weight	Lipid Amount (mg/ml)	Lipid percantage amount (%)
1	*Phyllobius karamanensis*	8 mg	0,636±0,25	7,95±0,56
2	*Oedecnemidius saltuarius*	6 mg	0,502±0,08	8,366±0,97
3	*Polydrusus spp.*	3 mg	0,100±0,01	3,333±0,25
4	*Curculio nucum*	15 mg	0,860±0,17	5,733±0,49
5	*Curculio glandium*	17.5 mg	3,527±0,53	20,154±1,14
6	*Curculio villosus*	5 mg	0,235±0,17	4,7±0,63
7	*Hypera postica*	10 mg	1,399±0,19	13,99±0,99
8	*Gymnaetron asellus*	12.5 mg	0,646±0,08	5,168±0,43
9	*Gymnaetron tetrunn*	4 mg	0,503±0,04	12,575±1,11
10	*Zacladus asperactus*	3.75 mg	1,945±0,54	51,866±1,27
11	*Lixus cardui*	41.4 mg	3,257±0,71	7,867±0,91
12	*Larinnus latus*	286 mg	0,475±0,06	0,166±0,17
13	*Baris spp.*	5 mg	0,244±0,04	4,88±0,65
14	*Sitona hunneralis*	5 mg	0,383±0,08	7,66±0,94
15	*Ceutorhynchus spp.*	2 mg	0,257±0,09	12,85±1,19

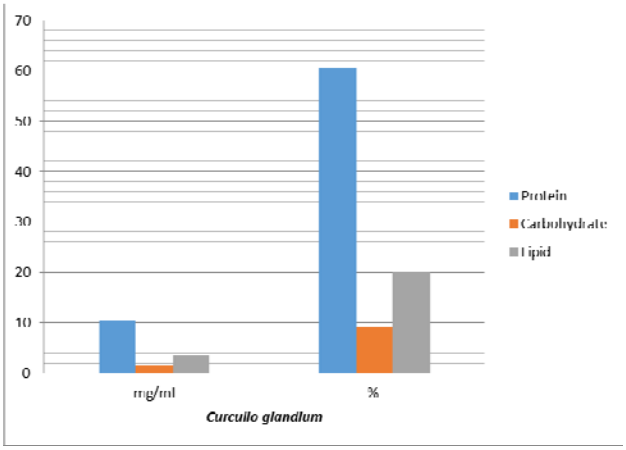
Table 4: Per individual total and percentage of protein amount

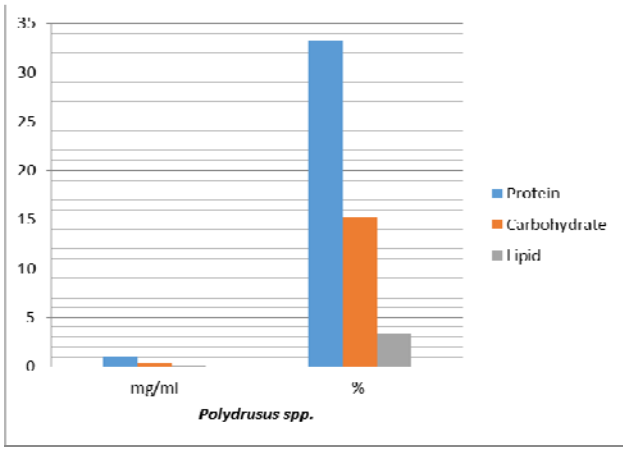
No	Species name	Per individual average Wet weight	Protein amount (mg/ml)	Protein percantage amount (%)
1	*Phyllobius karamanensis*	8 mg	1,718±0,3	21,475±0,12
2	*Oedecnemidius saltuarius*	6 mg	0,518±0,01	8,633±0,07
3	*Polydrusus spp.*	3 mg	0,998±0,04	33,266±0,61
4	*Curculio nucum*	15 mg	2,226±0,15	14,84±0,21
5	*Curculio glandium*	17.5 mg	10,563±0,23	60,36±1,16
6	*Curculio villosus*	5 mg	0,600±0,05	12±0.15
7	*Hypera postica*	10 mg	4,030±0,13	40,3±2,13
8	*Gymnaetron asellus*	12.5 mg	1,777±0,10	14,216±0,95
9	*Gymnaetron tetrunn*	4 mg	1,435±0,09	35,875±1,28
10	*Zacladus asperactus*	3.75 mg	5,357±0,08	143,33±2,16
11	*Lixus cardui*	41.4 mg	8,030±0,14	19,396±0,94
12	*Larinnus latus*	286 mg	1,632±0,19	0,570±0,09
13	*Baris spp.*	5 mg	0,675±0,06	13,5±0,17
14	*Sitona hunneralis*	5 mg	1,079±0,11	21,58±1,28
15	*Ceutorhynchus spp.*	2 mg	0,107±0,01	5,35±0,93

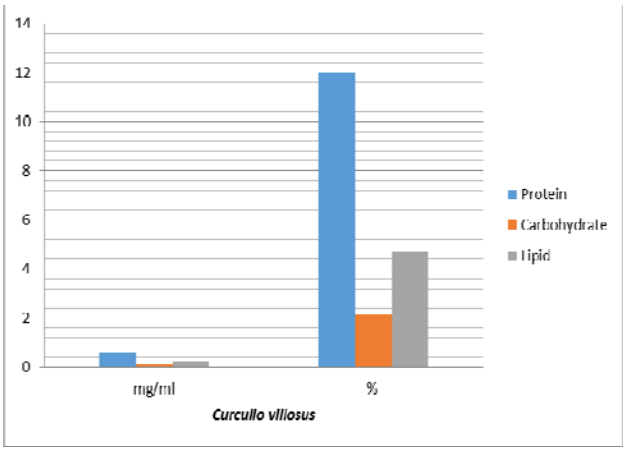


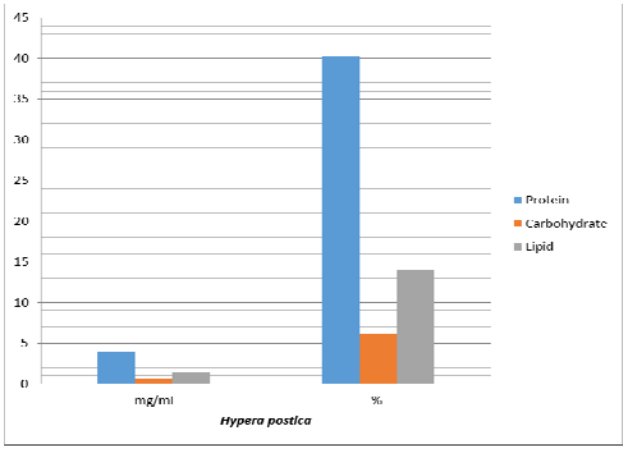








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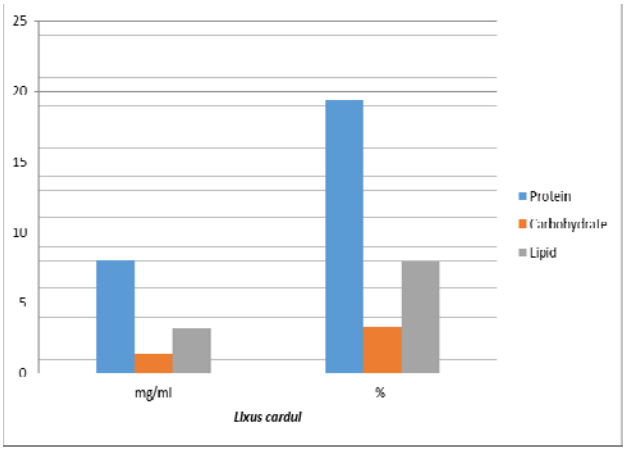
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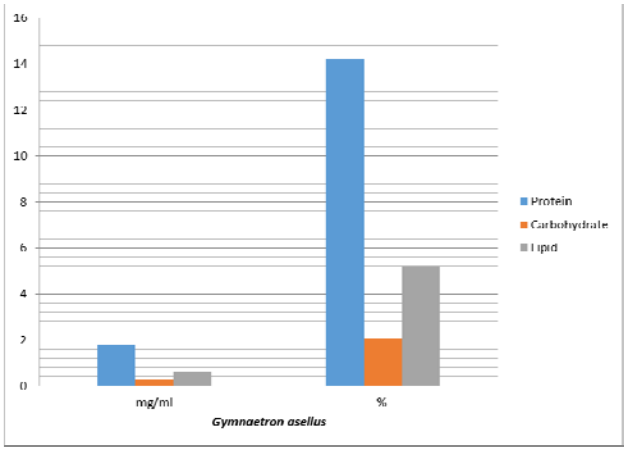
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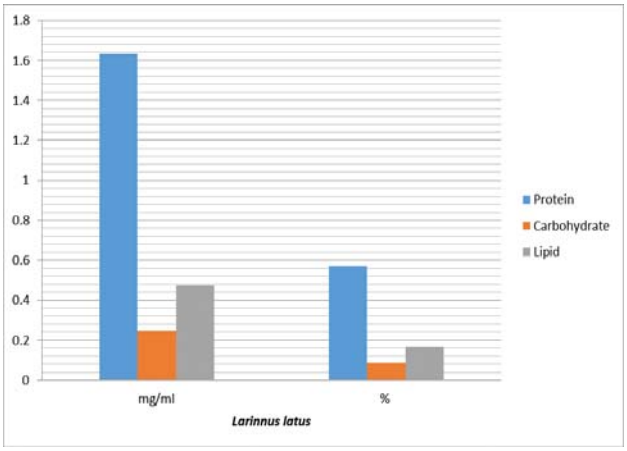
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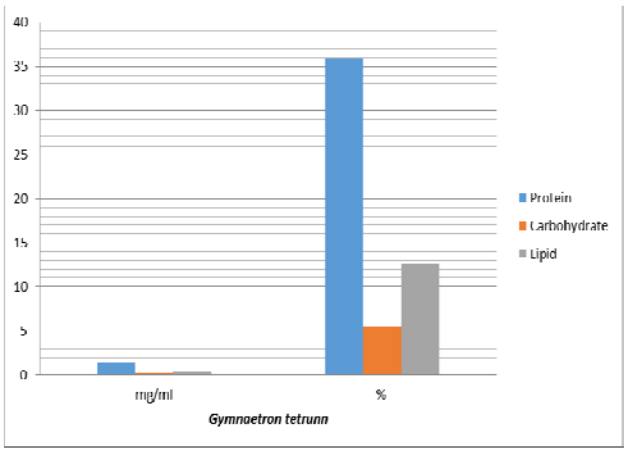
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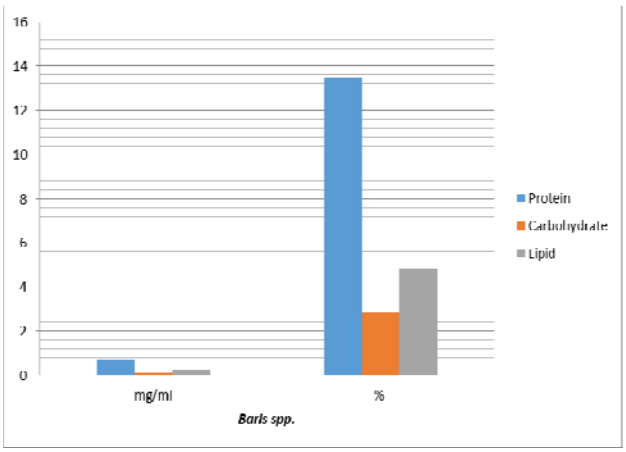
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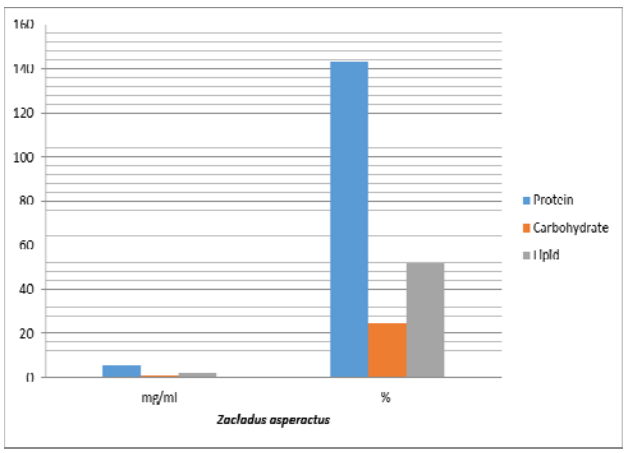
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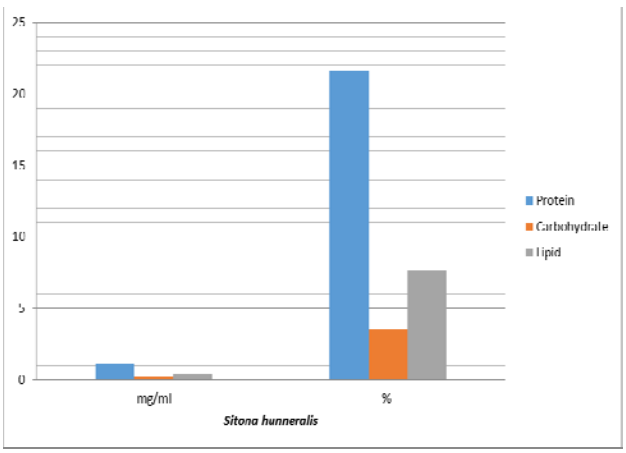
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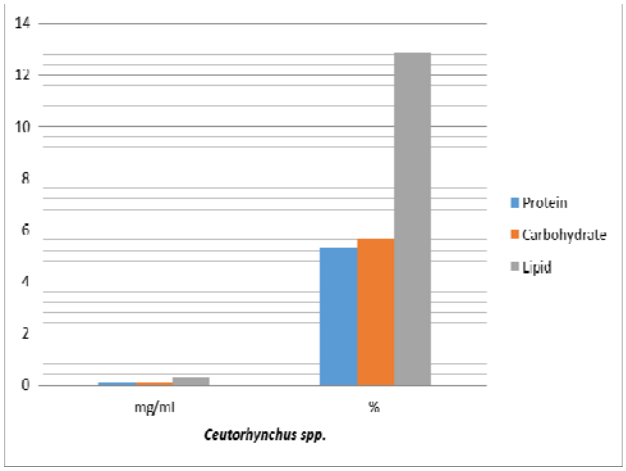

Fig 1: Protein, lipid and carbohydrate amount of each species treatmented

In this study; protein content was the most, carbohydrates content was the least in all species. Because protein is the component of structure, but carbohydrate is usually taken outs through nutrition.

4. Discussıon

In recent years, so many pest control methods is being growded in biological warfare. In other words biological control is the most important against harmful insects. In order to produce effectively control methods, biology, physiology, biochemistry are important to know.

Proteins are the most important organic constituents of animal tissues including insects and play an important role in energy production. In the development process, various agents such as protein is required for the synthesis of ATP (Taşkın and Aksoylar, 2011) [17].

In another study conducted to determine the effects of host density on development time, egg dispersion, fecundity, sex ratio, longevity, and glycogen, total sugar and lipid levels of *Bracon hebetor* Say, 1836 (Hymenoptera: Braconidae). As a result of research conducted that Host density had no significant effect on glycogen levels of female and male wasp, whereas sugar and lipid levels showed some variations in both sexes (Işıtan *et al.,* 2011) [4].

Lipids are used in so many insects as energy source, hormone precursors and structural members. It is stored in different regions in the insect body. Also lipids located in the egg play an important role in meeting the energy needs for developing embryo (Boz and Gülel, 2012) [1].

Fluctuation of lipid content in different insects species treated with so many toxicants has been reported by several researchers. Copuzzo and Lancaster (1981) [2] have shown an important decrease of lipid content in the fat body of *Homarus americanus* when exposed to toxicants. The similar trend has been observed in carbohydrate, protein and lipids by *Aspongopus janus* (Hemiptera) when treated with nimbeciline including 0.03% Azadirachtin Thiruvasagam, (1994) [18], *Periplaneta americana* when treated with *Pongamia glabra* leaf extract Ramanathan, (1995) [8] and *Laccotrephes ruber* when treated with monocrotophos Ravichandran (1996), *Gryllotalpa africana* when treated with endosulfan (Sumathi, 2001) [16], *Laccotrephes ruber* when treated with zinc, *Sphaerodema rusticum* when treated with mercury (Rajathi, 2004) [7]. All these results indicate that, proteins, lipids and carbohydrates are important.to selection of an effective biological control agents.

Several larvae feed on knapweed seeds and pupate in the capitula, making a cocoon out of the seed head material. Also, some larvae can destroy up to 100% of the seeds in a capitulum in the Curculionidae family (Story and White, 1996) [15].

Many examples of biological control studies mentioned above indicate that a detailed knowledge of the ecology and biochemistry including proteins, lipids and carbohydrates of Curculionidae necessary to achieve successful control.

Insect control strategies interfering with α amylases, and thus food digestion, are known to reduce insect survival and growth, and, for this reason, many studies have focused on biochemical studies including carbohydrates on Curculionidae (Riseh and Ghadamyari, 2012) [13].

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6. References

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