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BLACK CUMIN (*Nigella sativa* L.) SEEDS AS POTENTIAL FOOD ADDITIVE SOURCE

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ABSTRACT

Black cumin (*Nigella sativa* L.) seeds represent flavouring agent in cheese and backery products. Therefore, biochemical studies and biological assay were conducted. The results illustrated that both extracted proteins at pH 8.0 (24.85% protein) and protein concentrate at the isoelectric point pH 4.4 (68.15% protein) were fractionated by SDS-PAGE and the two extracted protein contained 14 and 11 subunits for the first and second, respectively. The molecular weights of polypeptide chains ranged from 94.5 to less than 12.0 kDa. Data of amino acids for both two protein extracts indicated that the presence of diffiency amounts in lysine and sulfur amino acids. On the other hand, biological assay showed that each body weight gain (BWG), protein efficiency ratio (PER) and digestibility coefficient (DC) of albino rats fed on *N. sativa* protein concentrate (N.S.PC) were significant decreased than groups fed on control and/or skimmed milk powder as protein source in diets. And thus it must be added any animal protein source to improve the protein quality of *N. sativa* seeds. However, these results are similar reported data for some legumes protein. On the other studies are concerning with extraction of natural products from *N. sativa* seeds were carried out. Methanolic extract contained antioxidant activity (AOA) for both crude extract (I) and purified compound (II). The results indicated also that AOA were 56.75 and 52.70% of the total activity of α-tocopherol as standard with respecting to control. These two extracts were used as antifungal against bread mold fungi (*Rhizopus stolonifer*). Data revealed that 10 and 50 μg/ml concentrations were effective against linear growth of fungi while 100 μg/ml was the most effective against bread mold fungi. However, it can be concluded that *N. sativa* L. seeds were represented potential food and feed additive source and good protective agent.

Keywords: Nigella sativa, Black cumin, Rhizopus stolonifer, protein efficiency ratio (PER).

INTRODUCTION

Black cumin (*Nigella sativa* L.) is cultivated in Egypt, and known under different Arabic name: Habbah soeda, kamum Aswan and Habbet El-Barak. The seeds possess aromatic odour and taste (Salama, 1973). The seeds are also used in the cooking and on bread products as a flavouring agent in Egypt, India and Turkey (EL-Komey,1996). Black cumin seeds have many medical uses such as digestive stimulants, carminative aromatic, diuretic, diphonetic stomachic and anthelmitec agents (Agarwol, *et al.*, 1979 and Ibrahim, 1997).

Protein and amino acid composition of *N. sativa* L. seeds have been reported by AL-Jassir (1992); Abdel Aal and Attia (1993) and Naroz (1997). On the other hand, antioxidative effects of black seeds were also discussed by Sosulski (1979), Nergiz (1991) and Nergiz and Otles (1993). Antioxidant substances are present in *N. sativa* L. seeds such as α, β and γ - tocopherols(about 340 μg/g) and polyphenols (about 1750 μg/g) in the seed oil. These compounds may be considered as native medicine and

flavouring agents in several foods. However, little informations are available about the significant role of black seeds in food ingredients and biological human systems.

The aim of study to evaluate extracted proteins from *N. sativa* L. seeds; electrophoretic patterns of the extracted protein (EP) and protein concentrate (PC) by SDS-PAGE; essential amino acid compositions, biological of the protein concentrate using experimental animals feeding; natural products were extracted from seeds act as antioxidant compounds, and using these extracts as antifungal substances to evaluate the black seeds as potential feed and food additive source.

MATERIALS AND METHODS

Nigella sativa L seeds were obtained from the Central, Agricultural Research, Giza, Egypt in 2002. The seeds were air dried and ground in a coffee grinder and stored at 4°C.

Biochemical analysis: -

The defatted matter was used to extract total protein (EP) by the method described by Tella and Ojehomon (1980). Solubility was carried out at different pH ranged from 2 to 12 using the method described by EL-Morsi, (1982). Protein concentrate (PC) was conducted by precipitating at pH 4.4 and redesolving using phosphate buffer solution at pH= 8.0 and then freeze-dried. Fractionation of both EP and PC were carried out by SDS-PAGE according to the method of Laemmli (1970). Densitometric scanning of gels with Coomassie Brilliant Blue R-250 were scanned at 580 nm, Jonior 24 with PC spectrophotometer equipped for scanning gel in dimension. Amino acids were determined using automatic amino acid analyzer model Alpha Plus 4151 LKB Biochrom at Central Laboratoy, Fac. of Agric., Cairo Univ. for both EP and PC. The amino acids score were calculated reffering to the provisional amino acids scoring pattern of FAO/WHO (1985).

Biological assay: -

The *in-vivo* protein quality evaluation was done by feeding the male of albino rats (age 4 weeks) with both diets of PC and/or mixture of (8% PC + 2% skimmed milk powder) as a protein source in the mixture diet . Five rats (weight ranged from 45 to 50 g) were assigned to each dietary treatment, fed adlibitum for 28 days and the results were compared with rats fed control (Casein) and skimmed milk powder diets. Table (1) showed the constituents of rat's diets. Body weight gain (BWG) and protein efficiency ratio (PER) were assayed according to the method of AOAC (1990) using this equation:

PER = Body weight gain (g)/ protein intake (g).

Digestibility coefficient was conducted according to Hsu, *et al* (1977).

Significant differences between means of 5 replicates were tested by T-test and one way ANOVA Spss package at $P < 0.05$.

Extraction and assay of antioxidants: -

The seeds of *N. sativa* L, were ground. About 5 g of ground matter was extracted with 50 ml methanol/distilled water (9:1) overnight, followed by filtration and evaporation to dryness under vacuum at 40°C. The final

obtained divided into two portions, the first one was represented crude extract (I) and the second was separated by gel filtration (Silica gel column 60x2 cm diameter) using solvent "methanol/water/ 1NHCl (90:9:1). The major component was fractionated on TLC. The major band was collected and redissolved in the same solvent and then filtrated and dried to obtain a purified compound (II).

Table (1): Constituents of rat 's diets

Constituents	G/100 g dry weight basis			
	Control	N.S. PC[1]	Mixture[2]	S. milk powder[3]
Starch	70.0	65.3	62.6	54.5
Casein	10.0	-	-	-
Protein source	--	14.7	17.4	25.5
Corn oil	10.0	10.0	10.0	10.0
Cellulose	4.8	4.8	4.8	4.8
Salt mixture	4.0	4.0	4.0	4.0
Vitamin mixture	1.0	1.0	1.0	1.0
Choline chloride	0.2	0.2	0.2	0.2

1.N.S.PC = Nigella sativa protein concentrate.
2. Mixture = Contained 8% net protein of N.S.PC and 2% net protein of skimmed milk powder.
3. Protein of skimmed milk powder = 39.22%.

Antioxidant activity (AOA) of two methanolic extracts (I and II) were determined using linoleic acid system (Osawa and Namiki, 1981). Each sample (200 μg) was added to a solution mixture of linoleic acid (0.13 ml), 99% distilled ethanol (10 ml) and 50 mM phosphate buffer (pH 7.0). The total volume was adjusted to 25 ml with water. The solution was incubated at 40°C and the degree of oxidation was measured according to method of Mitsuda, *et al.* (1966) by measuring the absorbance at 500 nm after colouring with $FeCl_2$ and thiocyanate. α-tocopherol (200 μg) was used as standard sample. Both two extracts (I and II) were stored in the dark at 4°C interval periods (0, 15, 30, 45, 60, 75 and 90 days). Each sample was measured as AOA%.

Bioassay antioxidant as antifungal agent: -

Bioassay of both I and II extracts were carried out against bread mold fungi (*Rhizopus stolonifer*) according to the method was described by Galal and Abdo (1996). Bread mold fungi were isolated from naturally infected local bread. The previous extracts were dissolved in ethanol to obtain the disered concentrations (10, 50 and 100 μg/ml) in order to test their direct effect on fungi. Mycelium disks (5-mm-diameter) from the growing edge of 3 day-old cultures of each treated fungus grown separately were inoculated onto these plates in order to test the effect of different concentrations of the two methanolic extracts on the linear growth of *Rhizopus stolonifer* fungi.

RESULTS AND DISCUSSION

Biochemical studies included extractability, solubility, and fractionation of *N. sativa* L. protein and characterization of these extracted protein hydrolyzates to evaluate their essential amino acids. The total extractable (EP) contained 24.85% crude protein. The solubility curve (Fig. 1)

showed that about 85.0% protein was soluble between pH 8 and 10. Minimum solubility (about 11.5%) occurred at pH 4.4 which is represented the isoelectric point. Similar results were reported by Naroz (1997) who indicated that the isoelectric point of *N. sativa* albumins and globulins were at pH 4.2 and 6.2, respectively. Protein concentrate (PC) was redissolved by phosphate buffer (pH 8.0) contained 68.15% protein. In addition, fractionation of both EP and PC were carried out by SDS-PAGE (Fig. 2). Densitometric analysis measured the intensity of protein band scanning each one through a visible light, therefore the densitometric analysis can provide simultaneous identification and quantification of subunits (Hou and Ng, 1995). Electrophoretic patterns showed the presence of 14 subunits with molecular weight ranged from 94.5 to less than 12.0 kDa, as well as 11 subunits having MWs ranged from 82.0 to 12.2 kDa for EP and PC, respectively. However, all informations of several protein bands were tabulated beside Fig. (2). Data indicated also that the highest percentages of the protein components were the 9th subunit had MW 25.0 kDa was 28.3% and the third one had MW 67.0 kDa was 23.1 for EP, whereas the highest one was the third had MW 53.5 kDa (26.1%) followed by the 8th one had MW 21.5 (25.1%) for Cp. However these differences may be due to the different solubility for these components among pH 8.0 and 4.4 (Tella and Ojhomon, 1980).

The results of amino acid composition of both EP and PC are shown in Table (2).

Table (2): Amino acid composition of *N. sativa* L. seeds

Amino Acids	Extracted protein (EP)		Protein concentrate (CP)		FAO/WHO (1985)
	g/100 g protein	Score %	g/100 g protein	Score %	
Essential A.A.					
Lysine	3.87	66.74	4.05	69.83	5.8
Met.+Cys.	2.05	89.13	1.98	86.09	2.3
Leucine	7.02	106.36	6.95	105.30	6.6
Isoleucine	4.11	146.79	4.25	151.78	2.8
Phe.+Tyr.	7.80	123.81	7.73	122.70	6.3
Valine	5.23	149.43	5.37	153.42	3.5
Threonine	4.27	125.59	4.33	127.35	3.4
Tryptophan	1.27	115.45	1.35	122.73	1.1
Nonessential A.A.					
Glutamic acid	17.95		18.10		
Aspartic acid	12.44		11.35		
Glycine	5.82		6.75		
Serine	4.21		4.13		
Alanine	4.88		5.05		
Arginine	6.59		6.84		
Histidine	3.90		3.83		
Proline	5.18		4.77		
T.E.A.A	35.62		36.01		
T.A.A.	96.59		96.83		
E/T ratio	36.88		37.19		
First limiting	Lysine		Lysine		
Second limiting	S-amino acids		S-amino acids		

Data showed that total essential amino acids were 35.62 and 36.01 g/100 g protein for EP and PC, respectively. Total essential/total amino acids (E/T ratio) were 36.88 and 37.19% for EP and CP, respectively. It is interest to notice that these ratios were more than recorded ideal protein (36%), although both of EP and PC had two limiting amino acids lysine and sulfur amino acids, respectively.

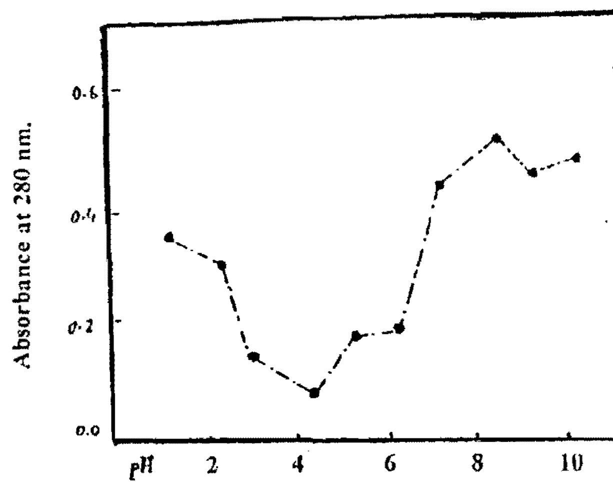
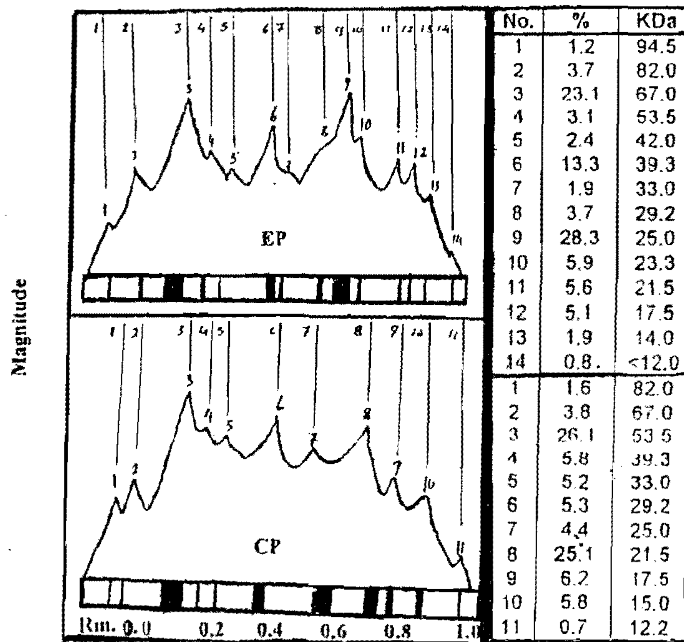


Fig (1): Effect of differnt pH on the solubility of protein extracted from *N. sativa* L. seeds .



No.	%	KDa
1	1.2	94.5
2	3.7	82.0
3	23.1	67.0
4	3.1	53.5
5	2.4	42.0
6	13.3	39.3
7	1.9	33.0
8	3.7	29.2
9	28.3	25.0
10	5.9	23.3
11	5.6	21.5
12	5.1	17.5
13	1.9	14.0
14	0.8.	<12.0
1	1.6	82.0
2	3.8	67.0
3	26.1	53.5
4	5.8	39.3
5	5.2	33.0
6	5.3	29.2
7	4.4	25.0
8	25.1	21.5
9	6.2	17.5
10	5.8	15.0
11	0.7	12.2

Fig (2) : Densitometric scanning of SDS-PAGE of extracted protein (EP) and protein concentrate (PC) from *N. sativa* L. seeds .

However, the diffiency of both lysine and sulfur ones can be supported with animal protein source. Similar results were reported by Babayan, *et al* (1978), Abdel Aal and Attia (1993) and Naroz (1997).

Biological assay: -

Gain in body weight of rats fed control, *Nigella sativa* protein concentrate, mixture and skimmed milk powder as sources of protein in diets are summarized in Table (3). The body weight gain (BWG) was increased with the increasing of age for all tested animals. However, the role of increasing of BWG was varied among tested groups.

Data indicated that BWG of protein concentrate (60.9±1.119) was less than recorded for control and dry milk (70.1±0.87 and 68.7±1.05 g, respectively). Consequently, PER of the tested protein concentrate (1.60±0.10) was also decreased than that recorded for control and dry milk (1.89±0.02 and 1.85±0.03, respectively). In addition, digestibility coefficient (DC) of *N. sativa* protein concentrate (N.S.PC) was available value (71.1±0.25) but it also less than DC of other two groups were mentioned previously. However, these results of BWG, PER and DC for rats fed N.S.PC. were significantly decreased when compared with control (casein) and skimmed milk powder. Therefore, it can be improving the protein quality of N.S.PC. by adding slightly amounts of dry milk to N.S.PC. in order to increase of growth (BWG), protein utilization (PER) and digestibility. And thus, the results of mixture between 8% net protein of N.S. PC. And 2% net protein of skimmed milk powder as total 10% protein source indicated that BWG, PER, and DC% were: 66.1±0.99, 1.82±0.07 and 78.9±0.16, respectively. It is interest to notice that it was very near for animal protein sources (Table 3).

Table (3): Mean values of body weight gain, protein efficiency ratio and digestibility of rat fed different experimental diets".

Protein source	Initial weight (g)	Final weight (g)	BWG (g)	Food intake (g)	Protein intake (g)	PER		DC
						Actual	Corrected	
Control	48.8 ± 0.51	118.9^{a} ± 1.10	70.1^{a} ± 0.87	370.2^{b} ± 1.04	37.0^{b} ± 0.12	1.89^{a} ± 0.02	2.50	85.3^{a} ± 0.27
S. milk powder	46.9 ± 1.51	115.6^{a} ± 0.35	68.7^{a} ± 1.05	370.9^{b}± 0.53	37.1^{b} ± 0.06	1.85a± 0.03	2.45	86.0^{a} ± 0.40
N.S.PC	49.3 ± 1.30	110.2^{b} ± 0.23	60.9^{c}± 1.11	381.0^{a} ± 2.03	38.1^{a} ± 0.21	1.60^{b} ± 0.10	2.12	71.1^{c} ± 0.25
Mixture	50.2 ± 1.08	116.3^{a}± 0.43	66.1^{b}± 0.99	367.1^{c} ± 1.96	36.7^{c} ± 0.23	1.82^{a} ± 0.07	2.41	78.9^{b} ± 0.16

. Mean values of five rats ± standard division, a, b, c indicate significant difference at $p<0.05$, DC = Digestibility coefficient

These improving values may be due to the complementary effect and protein utilization in the mixture diet induced the increasing of BWG and PER, as well as digestibility (Hassan and EL-Shewey (1999); EL-Malky and Kerolles (2000) and Abdel-Naem et al., (2003).

Antioxidant activity of *Nigella sativa* L. seeds: -

Antioxidants are principle ingredients that protect food quality be retarding oxidative breakdown of lipids (Madhavi and Solunke, 1995). The addition of antioxidant had become popular as a mean of increasing the self life of food products by 15~200% and improving stability of lipids and preventing of sensory and nutritional quality (Ghazy *et al.*, 2000).

Antioxidative activity (AOA) of crude methanolic extract (I) and purified compound (II) that extracted from *N. sativa* L. seeds in the linoleic acid system are shown in Fig. (3). Values are the average of three replicates had been done. A control containing no antioxidant or additive represented 100% lipid pyroxidation. The AOA of the two extracts (I and II) are compared with α-tocopherol as strong antioxidant (100% AOA). As shown in Fig. (3), methanolic extracts inhibited the lipid proxidation about 50,0 and 46.43% for I and II, respectively when comparing with control. In addition, the AOA of two methanolic extracts were 56.75 and 52.70% of the total activity of α-tocopherol for I and II, respectively with respecting of control. The stability characteristics of the two methanolic extracts were studied during interval storage periods in the dark at 4°C (90 days). The results are shown in Fig. (4) indicated that AOA increased from 56.75 to 70.90% and from 52.70 to 61.54% for extracts I and II, respectively at the end of 30 days. Gradually decreasing was observed at 45 days until 90 day. The final decreasing of AOA at the end of 90 days were 22.22 and 28.89% of the total activity of α-tocopherol for I and II, respectively. However, the storage under linoleic acid oxidation in the dark at 4°C illustrated that AOA of *N. sativa* L. seed extracts highly stable until the first 30 days, then gradually decreased until the end of storage periods. Similar results have been reported by Hayes, *et al.* (1977) and Shaker *et al* (1995).

In discussing sources for the AOA in methanolic extracts (I and II), it could be suggested that the potent activity may be due to the presence of phenolic compounds in *N. sativa* L. seeds (Chimi *et al* 1991). In addition, protein hydrolyzate and aromatic amines, as well as sulfhydryl compounds posses antioxidant activity (Hayes *et al* 1977).

Inhibitory effect of antioxidants against bread mold fungi: -

Inhibitory effect of antioxidants of the two methanolic extracts (I and II) on linear growth of bread mold fungi (*Rhizopus stolonifer*) after incubated 3 days at 25±2°C in dark by using three concentrations (10, 50 and 100 μg/ml are shown in Table (4) and Fig. (5). Data indicated that inhibitory effects were 78.7±0.37, 60.9±1.88 and 20.8% for crude extract (I) at the same order of the three concentrations, respectively, while purified compound (II) inhibited the linear growth of bread mold fungi (77.2±1.15, 64.3±1.73 and 18.5%) at 10, 50 and 100μg, respectively. However, the inhibitory effect increased with the increasing concentration for both extracts and the last concentration 100 μg/ml was the most effective on linear growth of *R. stolonifer* (about 80% comparing with control) for I and II extracts, individually (Table 4). Therefore, it can be concluded that methanolic extracts of *N. sativa* L. were represented antifungal agent. However, similar results using *N. sativa* L. seeds as sources of antimicrobial compounds were reported by EL-Alfy *et al* (1975).

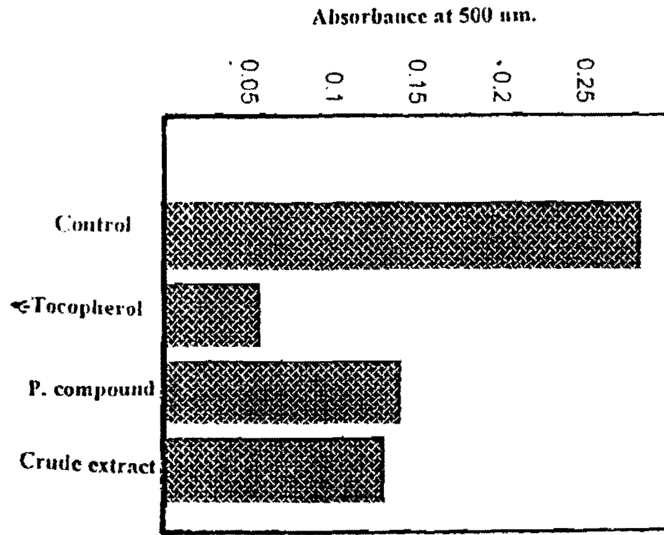


Fig (3) : Antioxidative activity of crude methanolic extract (I) and purified compound (II) of *N. sativa* L. seeds .
Control represented 100 % lipid pyroxidation .

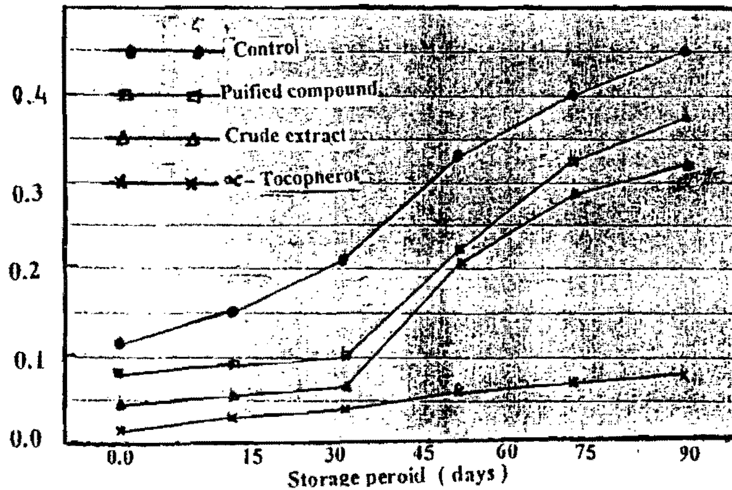


Fig (4) : Effect of storage peroids on AOA of methanolic extact and purified compound extracted From *N. sativa* L. seeds

Table (4): Inhibitory effect of extracted antioxidants from *N. sativa* L seeds against bread mold fungi.

Extracts (µg / ml)	10	50	100
Control	83.1±0.36[a]		
α-Tocopherol	72.8±0.76[c]	51.4 ±0.85[b]	N.D
Crude extract	78.7±0.37[b]	60.9 ±1.88[a]	20.8
Purified compound	77.2±1.15[b]	64.3±1.73[a]	18.5

N.D = Not detected
Mean ±S.D. at P< 0.05, a, b, c indicated significant differences

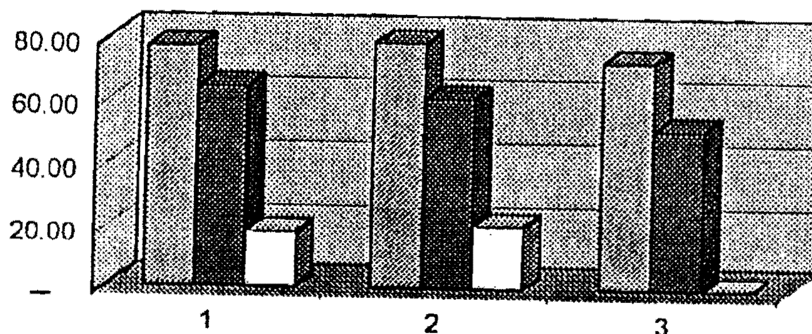
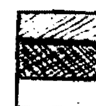


Fig.(5) : Antioxidants of *N. sativa* L. seeds as antifungal agent.

1. Purified compound
2. Cude extract .
3. α– Tocopherol.



10 ug / ml.
50 ug/ ml..
100 ug / ml.

In conclusion, from all the previously results it can be revealed that *N. sativa* L. seeds were represented a good food additive source with other slightly addition of one animal protein source such as dry milk because *N. sativa* proteins were deficient in the essential amino acids (lysine and S-amino acids). In addition, single *N. sativa* proteins were less values of BWG, PER and DC than control and skimmed milk powder and similar to those values of some legume proteins sources. Therefore, it should be supported by little amounts of one source animal protein to become a potential food protein source.

Data revealed that the presence of antioxidant activity in the methanolic extracts of *N. sativa* L. seeds, so these extracts have many medical properties and increase the immunity and maintaining good health. In addition, these antioxidants act as antifungal agent against bread mold fungi particularly at the high concentration (100 □g/ml). However, *N. sativa* L. seeds are represented a good food additive source for many foods, particularly backery products. Further studies must be carried out using several natural products extracted from black cumin seeds for both fat and water soluble ones to release a good health as food additive source and potential protective agent.

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"بذور حبة البركة السوداء كمصدر غذائى إضافى وصحى قوى"

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تعتبر بذور حبة البركة السوداء من مكسبات الطعم لأغذية عديدة فى صناعة الجبن والمخبوزات لذلك أجريت دراسات كيميائية حيوية وبيولوجية عليها وأوضحت النتائج أن البروتين المستخلص على (pH 8.0) كانت نسبة البروتين ٢٤,٨٥% ومركزات البروتين على (pH 4.4) كانت ٦٨,١٥% وعند تفريده كهربيا بواسطة SDS-PAGE أعطى ١٤ و ١١ سلسلة ببتيدية للأول والثانى على التوالى وكان وزنها الجزيئى محصورا بين ٩٤,٥ إلى اقل من ١٢ كيلو دالتون و أوضحت دراسة تحليل الأحماض الأمينية أن كلا المستخلصين ينقصان فى الليسين والأحماض الكبريتية (ميثيونين وسيستينين) كأحماض امينية أساسية. كذلك أوضحت انه عند إضافة مركزات البروتين كمصدر بروتينى وحيد فى تغذية فئران الالبينو لمدة ٢٨ يوم ومقارنتها بعليقة كنترول (كازين) وأخرى لبن جاف (كمصدر بروتينى) أن الزيادة المكتسبة فى الوزن (النمو) ، ومعامل كفاءة البروتين PER ونسبة الهضم قد نقصت معنويا عند مقارنة البروتين المختبر بالكازين أو اللبن ولكنها متقاربة مع بروتينات البقوليات :ومن هنا يتضح أن مركزات بروتين حبة البركة تصلح أضافتها كمدعم لمصادر بروتين أخرى لكى تصلح كغذاء عالى القيمة. هذا وقد استكملت دراسة بذور حبة البركة السوداء كمصدر إضافى وعلاجى لما يحتويه مستخلص الميثانول للبذور من مضادات الأكسدة أكدت النتائج أن كلا من المستخلص الميثانولى (١) والمركب النقى والذي لم يعرف تركيبه بعد (٢) لهما نشاط مضاد للأكسدة يقدر بـ٥٦,٧٥ ، ٥٢,٧٠ % من النشاط الكلى للالفا توكوفيرول القياسى (٢٠٠ ميكروجرام) مقاسا بالنسبة للكنترول لكلا من (١) ، (٢) على التوالى . كما أكدت النتائج أن مركبات مضادات الأكسدة المستخلصة من حبة البركة لها ثبات عالي حتى ٣٠ يوم من التخزين فى الظلام على درجة ٤°م .

وقد أستخدم المستخلص الخام والمركب النقى كمضاد فطرى ضد فطر عفن الخبز بثلاثة تركيزات (١٠،٥٠،١٠٠ ميكروجرام) وكان التركيز الأخير هو أشدها تثبيطا لنمو الفطر كـ linear growth بحوالى ٨٠% مقارنة بالكنترول.

من كل ما سبق يتضح أن بذور حبة البركة تعتبر كغذاء مدعم وكمصدر إضافى ووقائى لأغذية صحية تصلح كغذاء أدمى أو كإضافة غذائية ووقائية لأعلاف الحيوان.