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ORIGINAL ARTICLE **OPEN ACCESS**

Nutrients composition, amino acids and minerals content of *Nigella sativa L.* cultivated in, Eastern Uttar Pradesh (INDIA)

Dinesh Kumar Sharma*[1], Harish Sarswat[1]& Swapnil Rai[2]
1Mangalayatan University, Aligarh (U.P) INDIA
2 Amity University, Gwalior (M.P) INDIA
*e-mail. sharmadkchem@gmail.com

ABSTRACT
The nutritional value, proximate analysis, vitamins and amino acid composition of Nigella sativa L. seeds, cultivated in three different regions Kanpur, Faizabad and Bharaich in East Uttar Pradesh (India) were analyzed. Proximate analysis of Nigella sativa L. seeds showed that moisture contentswere3.7 ± 0.2, 4.0 ± 0.31 and 3.5 ± 0.02 % for Kanpur, Faizabad and Bharaich respectively. The crude protein was found 24.1 ± 0.53, 24.8 ± 0.17 and 23.5 ± 0.24 % for Kanpur, Faizabad and Bharaich site respectively. Magnesium, phosphors and calcium were the predominant elements present. Sodium, iron and zinc were found at lower levels. Thiamin and riboflavin were found while ascorbic acid was not found in Nigella sativa L. seeds. Glutamic acid, aspartic acid and arginine were the main amino acid present while cystine and methionine were the minor amino acids. These results showed that Nigella sativa L. seeds cultivated in India were found rich source of protein and nutrition.
Key Words: Nigella sativa L.,amino acid, minerals

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INTRODUCTION

Nigella sativa L. is commonly known as black seed. *Nigella sativa L.*is an annual erect herbaceous plant belonging to the Ranunculaceae family native to southwest Asia. It grows in countries bordering the Mediterranean Sea, Pakistan and India[1-3]. Nigella sativa L. locally known as "Kalonji" is good source of nutritionally essential components [1,4,5]. It is cultivated in India mainly Uttar Pradesh, Madhya Pradesh, Gujarat and Rajasthan state. The average length of plants is 20-30 cm, with finely divided, linear leaves (but not thread like). The flowers are delicate and usually pale blue and white colored with 5-10 petals. The fruit is a large and inflated capsule composed of 3-7 united follicles,each contains numerous seeds.

The oil of *Nigella sativa L*. seeds is considered as one among newer sources used for cooking. They are used for edible and medicinal purposes in many countries including India.They are used as a condiment in bread and other dishes. They are also used in the preparation of a traditional sweet dish, composed of black cumin paste, which is sweetened with honey or sugar syrup, and in flavoring of foods, especially bakery products [6-9].

*Nigella sativa L.*plant is one of the most extensively studied, both phytochemically and pharmacologically. Various researcher reported that *Nigella sativa L.* seed's oil possess antioxidant, anti-inflammatory, antitumor and antibacterial activity [10-14] besides their numerous folk medicinal usages such as treatment of influenza, eczema, headache, bronchitis, asthma, cough, fever, kidney and liver disorders, and as a diuretic, lactagogue, carminative and vermifuge[15].

The nutritional and composition values of any plant vary with the geographic conditions. Since there was no study has been carried out to investigate the minerals contents, amino acids and nutritional values of *Nigella sativa L*. seeds cultivated in Utter Pradesh (India). Hence keeping the above in view the present work was to determine the minerals contents, amino acids and nutritional values of *Nigella sativa L.* seeds cultivated in three different regions of east Uttar Pradesh in India.

MATERIAL AND METHODS

Reagents and samples

All the solvents were of Analytical Grade and were purchased from Rankem (India).Glacial acetic acid, meta-phosphoric acid, sulphuric acid, nitiric acid and perchloric acidwere also purchased from Rankem (India)sodium heptanes sulphonate, sodium acetate, potassium acetatea-amylase.Analytical grade

riboflavin, thiamine and L-ascorbic acid and amino acids reference standard were purchased from Himedia (India).*Nigella sativa L*. seed are collected from three different regions of Uttar Pradesh (India) namely Kanpur, Faizabad and Bharaich districts. Thoroughly washed *Nigella sativa L.* seed were dried in air oven at 60 ^{o}C for 72 h for further use. For HPLC analysis, Millipore water was used throughout the studies.

Instrumentations

Mineral nutrients in *Nigella sativa L*. seeds were analyzed using a Perkin–Elmer A- Analyst 800 atomic absorption spectrometer by suitable hollow cathode lamps after the digestion of ash of seeds using HNO_3, H_2SO_4 and $HClO_4$ acid and diluting with double distilled water to a specific volume.

Vitamins (riboflavin, thiamine and ascorbic acid) and amino acids were analyzed using reverse phase high performance liquid chromatography using waters HPLC system. The HPLC system consists of water 1525 binary HPLC pump and 717plus auto sampler (waters®). The chromatographic peaks of amino acids were identified and quantified using Breeze™ software (Version3.2).cAmino acids were analyzed AccQ Tag™ reverse phase (3.9×150 mm) 4 µm analytical column equipped with 2475 multi fluorescence detector (emission and excitation wavelength 395 and 250nm).Cystine and Methionine were analyzed from the same method of acid hydrolysis after treatment using performic acid oxidation. Vitamins (riboflavin, thiamine and ascorbic acid) were analyzed using an octadecyl end capped RP-C18 column (4.6 mm i.d. ×25 cm) 5 µm pore size equipped with a UV detector.

Preparation of standard solution

Standard solution of Ascorbic acid was prepared by dissolving 50 mg of ascorbic acid in meta-phosphoric acid (0.3 M) and acetic acid (1.4 M) solution at the final concentration 1mg/ml. Standard solution of riboflavin was prepared by dissolving 50 mg riboflavin in doubly distilled water followed by addition of three to four drops of glacial acetic acid and the solution was warmed to 85^{o}C. Final concentration of the riboflavin was made to100 µg/ml where as the standard solution of thiamine was prepared by dissolving 26.7 mg of thiamine hydrochloride in 25 ml of doubly distilled water.The stock and standard solution of amino acids were prepared in mobile phases.

Chromatographic conditions

Many analytical methods have been reported by various researchers for the determination of thiamine riboflavin and ascorbic acid [16-18]. Selection of method generally depends upon accuracy, sensitivity and the interferences encountered in the sample matrix. Thiamine, riboflavin, and ascorbic acid were identified by comparing the retention time of the sample peak with that of the thiamine, riboflavin, and ascorbic acid standard at 250, 270and 254nm. Quantification was carried out using external standardization. For the identification of thiamine, riboflavin mobile phase (12.5mM sodium acetatein a mixture of methanol/ water 25/75 +2.5mMsodium heptanes sulphonate) with a flow rate of 1.0 ml/min was used while for the identification of ascorbic acid mobile phase (0.1M potassium acetate pH 4.9 in a mixture of acetonitrile water 50/50) with a flow rate of 1.4 ml/min was used.

Sample preparation for analysis of trace elements

A 50.0 g of *Nigella sativa L.*seeds were crushed, grinded and powdered in a mortar. Dry ashing method was adopted by placing the properly dried sample in to the versatile crucible overnight in an electric muffle furnace maintaining the temperature between 400-440 °C. This ashing will destroy all the organic material from the sample. The ash was removed from crucible and dried in desiccator. The yield of ash was approximately 6 gm/ 50 gm. 1 gm of ash was taken and digested using conc. HNO_3, H_2SO_4 and $HClO_4$ in the ratio of 10:6:3. Digest was stored in sterilized bottles and used for the determination of Na, Mg, Ca, Zn, Fe and P on flame atomic absorption spectroscopy. Phosphorus was analyzed with colorimeter using ammonium vanadate-molybdate method. Three replicates were prepared for the each of the sample.

Sample preparation for analysis for vitamins

Riboflavin and thiamine were extracted using the method described in literature [19]. One gram of *Nigella sativa L.*seeds powder was transferred into a 50 ml graduated polypropylene centrifuge tube and followed by the addition of 20.0 ml of 0.1 H_2SO_4. The mixture was shaken vigorously for 1 min, and then placed in boiling water for 30 min and shaken at 5 min intervals. Now the mixture was cooled in an ice bath and followed by the addition of 2.5 ml of 2% a-amylase. After mixing properly, the mixture was incubated at 50°C for 1 hr in a water bath with shaking. The mixture was cooled and then diluted to 25 ml with deionised water. The resulting mixture was centrifuged. The supernatant was filtered through a 0.45 µm nylon filter disc before HPLC analysis. All samples were carried out in triplicates.

Vitamin C was extracted using the modified method of Abdulnabi *et al*. [20]. The One gram of *Nigella Sativa L.* seeds powder was homogenized with an extracting solution containing meta-phosphoric acid (0.3 M) and acetic acid (1.4 M). The mixture was placed in a conical flask (wrapped with aluminum foil) and agitated at 100 rpm with the aid of an orbital shaker for 15 min at room temperature. Mixture was

then filtered through a Whatman filter paper No. 4 to obtain the clear extract. The sample to extraction solution ratio was 1 to 1. All samples were extracted in triplicates.

Sample Preparation for analysis for amino acids

The sample was hydrolyzed in triplet using 6N HCl at 110^{o}C for 24 h and derivatized using AccQ reagent (6'Aminoquinol-N-hydroxysuccinimdyl carbamite) [21].

Proximate Analysis

Moisture content of *Nigella sativa L*. seeds was determined according to an air-oven method. Ash content was determined by incinerating at 410-440 ^{0}C until the constant weight was achieved. Total nitrogen and the protein content were determined based on the Kjeldahl method using the conversion factor of 6.25. All the above determinations were based on the methods of AOAC[19].

RESULT AND DISCUSSION

Proximate analysis

The Proximate analysis of the three different samples of *Nigella Sativa L.* seeds were carried out and result is presented in table 1 along with the comparison of these results with the literature. The percentage of moisture at the site-II (Faizabad) was found to be 4.0 ± 0.31, which is slightly high in comparison to Site-I (Kanpur) and Site-III (Bharaich) at which it was found 3.7 ± 0.2 and 3.5 ± 0.02 respectively. The percentages of crude protein were found 3.5 ± 0.02, 3.5 ± 0.02 and 23.5 ± 0.24 at the Site-I (Kanpur), site-II (Faizabad) and Site-III (Bharaich) respectively. The percentage of crude protein at the site-II (Faizabad) is slightly high in comparison to other two sites. These differences may be due to variations in environmental factors such as soil and irrigated water. The percentage of ash was found 5.0 ± 0.43 at Site-III (Bharaich) which is high in comparison to other two sites. These results differed slightly in moisture; ash and protein from those were reported in literature.

Table1. Proximate of *Nigella sativa L.* seeds; means of three determinations ±SD

Proximate composition (%) Nigella Sativa L. seeds from India				Values reported in literature				
	Site-I (Kanpur)	Site-II (Faizabad)	Site-III (Bharaich)	Syria [22]	Sauadi Arabia[23]	Turkey [24]	Jordon [25]	Yemen [26]
Moisture	3.7 ± 0.2	4.0 ± 0.31	3.5 ± 0.02	3.6	4.6±0.45	6.4±0.15	4.0	6.8 ±0.3
Ash	4.8 ± 0.11	4.5 ± 0.13	5.0 ±0.43	4.7	4.4 ±0.32	4.0 ±0.29	4.2	3.8 ±0.1
Crude Protein (N×6.5)	24.1 ± 0.53	24.8 ± 0.17	23.5 ± 0.24	20.9	20.9 ±1.35	20.2 ±0.82	19.9	20.0 ±0.7

Minerals analysis

The analysis of trace minerals such as Na, Mg, Ca, Zn and Fe were done by using atomic absorption spectroscopy. These minerals content of *Nigella sativa* L. seeds is essential in human nutrition. The mineral composition of three different samples of *Nigella sativa* L. seeds are shown in table 2 and Figure1. Trace metals uptake in vegetables plants and fruits absorbed from the soil of the cultivated area, the atmospheric condition and partly from the irrigated water.

Iron is one of the essential metals needed in various enzymatic reactions and its daily requirement is ranged 1.5-2.2 mg/day [27]. Iron content in Nigella sativa seeds ranged from 7.68 ± 0.08 to 10.2 ± 0.24 mg/ 100gm.Maximum iron content was found at site-I Kanpur while minimum was noted at site-II(Bharaich). Iron content analyzed in the present study was similar to that of Nigella sativa L. seeds cultivated in Yemen, Syria and Jordon [22,25,26]. In Saudi Arabia iron was found in very low amount0.15 mg/100g while it was found very high57.5 mg/100gin Turkey [23,24].

Table 2. Mineral content of three different samples of *Nigella sativa L.*seeds cultivated in India and some reported literature values; means of three determinations ±SD

Elements	Values of *Nigella sativa* seed from three Indian sites mg/100g			Values reported in literature				
	Site -I (Kanpur)	Site-II (Faizabad)	Site-III (Bharaich)	Syria [22]	SaudiArabia [23]	Turkey [24]	Jordon [25]	Yemen [26]
Na	54.66±1.69	46.96±0.94	57.53±0.91	53.50	0.75	85.30	41.9	44.00
Mg	202.16±2.78	119.62±2.01	268.52±1.51	-	0.03	-	-	219.00
P	460.36±10.70	432.90±2.29	472.93±1.99	569.90	1.80	526.70	502.3	65.00
Ca	193.20±1.93	146.70±1.51	212.76±1.98	200.50	0.04	188.50	186.7	544.00
Fe	10.20±0.24	8.38±0.13	7.68±0.08	9.30	0.15	57.50	10.7	8.60
Zn	7.68±0.08	6.52±0.08	5.16±0.13	5.90	0.09	5.60	5.9	1.84

Each value represents the mean of three replications ±SD

According to the WHO recommendation fruits and vegetables are poor sources of Zn and ranged up to 1 mg/kg and dietary intake for Zn is 14-20 mg/day [28]. Nigella sativa L. seeds are the rich source of zinc. Zinc content in Nigella sativa L. seeds cultivated in India ranged from 5.16 ± 0.13 to 7.68 ± 0.08.Results presented in table 2 shows that zinc content was found maximum at site-I (Kanpur) while minimum at site-III (Bharaich). The amount of zinc content is almost comparable with the *Nigella sativa L.* seeds cultivated in Turkey, Syria and Jordon while in Saudi Arabia and Yemen [22-26], it was found in very low 0.09 and 1.84 mg/100g.

Na as essential macro element has physiological effect in human and animal cellular and metabolic mechanism. The increased level of Na contents has direct link to the high blood pressure [29]. The Na daily recommended range in developing countries is between 2400-5175 mg/day [30]. Sodium content in *Nigella sativa L.* was found highest at site-III (Bharaich) 57.53 ± 0.91 while minimum at site-II (Faizabad) 46.96 ± 0.94 mg/100g. In Saudi Arabia sodium was found very low amount 0.75 mg/100g.

Calcium uptake in Nigella sativa L was higher *i.e*212.76 ± 1.98 mg/gm at site-III (Bharaich) while lower 146.7 ± 1.51mg/gm at site-II (Faizabad) showed lower Ca contents. Calcium is essential for teeth and healthy bones [31]. The health of the muscles and nerves depends on calcium. It controls the membrane structure, membrane permeability and provides the stability to cell [32]. The recommended daily allowance for Ca is for children between 500 and 1000 mg and for adults 800 mg [33]. Hence seeds of *Nigella sativa* L. was found good source of calcium. The balance of phosphorus and calcium is regulated by parathyroid hormone, which increases urinary excretion of phosphate under conditions of high phosphate and low calcium intake [34].

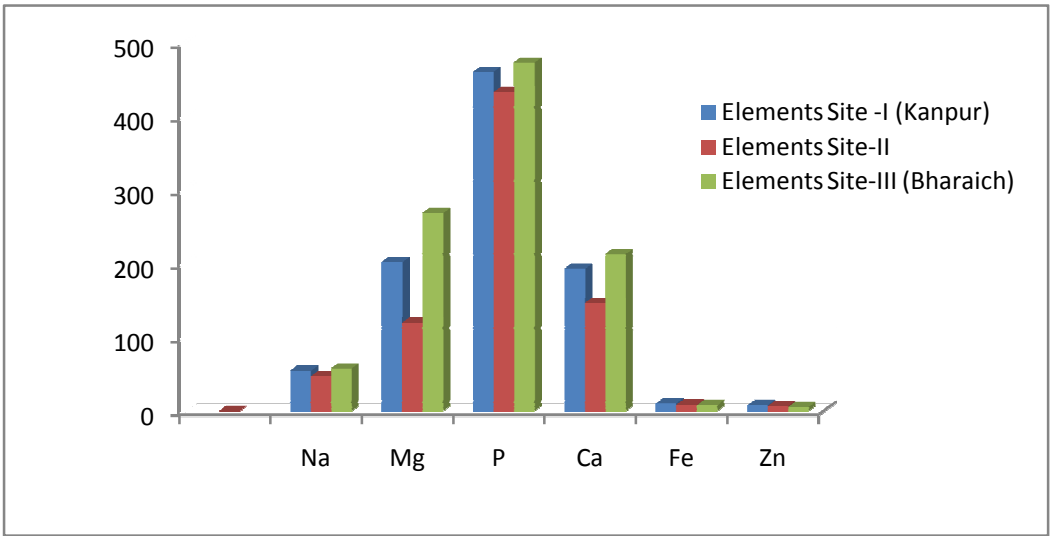

Figure 1 Elements in *Nigella sativa L.* seeds

Recommended Dietary Allowances have been set at 460–1250 mg of phosphorus per day for different age groups by the United States Institute of Medicine [35]. The amount of phosphorus found in the range between 432.9 ± 2.29 mg/100gm to 472.93 ± 1.99 mg/100gm. the high phosphorus concentration found at site-III (Bharaich) while site-II (Faizabad) showed low phosphorus concentration. In Saudi Arabia and Yemen concentration of phosphorus was found to be very low [23,26].

Magnesium is an essential mineral for human nutrition. It serves several important functions such as contraction and relaxation of muscles, function of certain enzymes in the body, and production of protein in the body. Magnesium daily dietary intake ranged 400-420 mg/day [36]. Nigella sativa L. cultivated in India was found to be good source of magnesium. The amount of magnesium found in the range between 119.62 ± 2.01mg/100gm to 268.52 ± 1.51mg/100gm. The results presented in table 2 showed that magnesium content was found maximum at site-III (Bharaich) while low at the site-II (Faizabad).Magnesium content found *Nigella sativa* L. seeds cultivated in India in is close agreement with the seeds cultivated in Yemen while in Saudi Arabia it was found to be very low amount 0.03 mg/100gm [23,26].

Vitamins analysis

The water-soluble vitamins Thiamin, Riboflavin and Ascorbic acid in *Nigella sativa seeds* were analyzed using HPLC technique comparing the peaks of vitamins with the standard. The concentration of Thiamin, Riboflavin and Ascorbic acid *in Nigella sativa L. seeds*, collected from different sample sites are

represented in table-3 and Figure 2. The result showed that ascorbic acid content in *Nigella sativa* seeds was found to be absent in the all samples collected from all three sites.

Table3: vitamins composition of *Nigella sativa L.* seeds; means of three determinations ±SD

S.No.	Vitamin	Site-I(Kanpur)	Site-II (Faizabad)	Site-III (Bharaich)
1	Thiamin	1.52 ± 0.03	1.58 ± 0.03	1.50 ± 0.01
2	Riboflavin	0.13 ± 0.01	0.11 ± 0.01	0.13± 0.01
3	Vitamin-C	-	-	-

Thiamin intake through supplement is 2.4 mg per day for man and 3.2 mg for woman. Approximately 27% of adults took Thiamin supplements to recover deficiency of Thiamin [37]. The amount of Thiamin (vitamin B_1) was found highest at site-II (Faizabad) and minimum at Site-III (Bharaich). At site-II (Faizabad), it was found to be 1.58 ± 0.03 while at Site-III (Bharaich) found to be 1.50 ± 0.01.

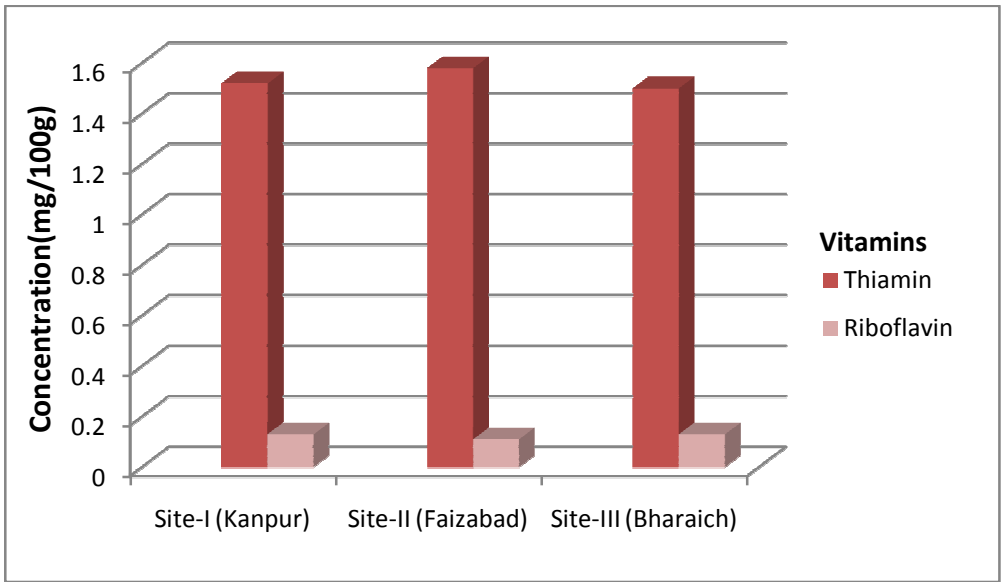

Figure 2 Thiamin and Riboflavin vitamins in *Nigella sativa L.* seeds

The amount of Riboflavin (vitamin B_2) was found to minimum at Site-II (Faizabad) while at Site-I (Kanpur) and Site-III (Bharaich) was found to be same which was 0.13 ± 0.01. The relative geographical representation of these vitamins according to their sampling sites is shown in figure-2.Recommended dietary allowance for Riboflavin for adult is 1.3 mg per day form man and 1.1 mg per day for woman [38].

Amino Acid

Amino acid profile of India's black cumin seed is represented in table 4 and figure 3. The protein consists of seventeen amino acid namely Leucine, Valine, Lysine, Threonine, Phenylalanine, Isoleucine, Methionine, Histidine, Alanine, Arginine, Aspartic acid, Cystine, Glutamic acid, Glycine, Proline, Serine and Tyrosine. In which first eight amino acids are essentials amino acids where as last nine were non essentials amino acids. *Nigella sativa L.* seeds contained 9303, 9770 and 9844 mg/100gm total amino acids at site-I (Kanpur), site-II (Faizabad) and site-III (Bharaich) respectively while essential amino acids were found 37.76, 37.93 and 37.55 of at site-I (Kanpur), site-II (Faizabad) and site-III (Bharaich) respectively. In the investigated material, the dominant amino acids were found glutamic acid (19.13% of the total content of amino acids) and arginine (9.95%) at site-III (Bharaich). On comparison, it was found that amounts of amino acid present in *Nigella sativa L.* seeds are close agreement with the literature.

Table 4 Amino acid compositions of Indian black cumin seed protein mg/100g, *mg/g

S.No	Amino acids	Site-I (Kanpur)	Site-II (Faizabad)	Site-III (Bharaich)	Ethiopia[b]* [39]	Syria[b]* [39]	Saudi Arabia[a][23]
	Essential amino acids						
1	Leucine	746±3.40	821±2.62	815±4.11	13.0 ±0.3	13.7 ±0.4	665±3.51
2	Valine	717±8.99	745±6.64	776±3.39	12.7 ±0.5	12.0 ±0.4	527±3.28
3	Lysine	459±2.94	478±4.32	472±2.05	9.3 ±0.2	8.8 ±0.4	462±4.28
4	Threonine	307±5.24	320±1.63	320±3.68	8.4 ±0.2	7.7 ±0.1	417±3.31
5	Phenylalanine	504±4.01	541±5.79	505±4.08	7.6 ±0.4	6.9 ±0.4	413±2.67
6	Isoleucine	343±2.94	358±3.40	341±1.24	9.0 ±0.2	9.6 ±0.3	395±2.11
7	Methionine	132±2.16	144±3.30	150±1.63	2.1 ±0.2	2.7 ±0.1	188±0.37
8	Histidine	295±4.08	305±0.47	318±2.16	5.5 ±0.2	5.5 ±0.3	383±1.64
	Non essential amino acids						
9	Alanine	379±2.05	409±2.05	391±3.86	9.0 ±0.2	9.6 ±0.3	427±3.35
10	Arginine	922±4.03	939±2.94	980±5.73	18.3 ±0.5	19.9 ±0.7	1051±10.39
11	Aspartic acid	898±5.88	897±2.05	916±3.26	15.8 ±0.5	16.0 ±0.5	1022±9.80
12	Cystine	150±4.08	162±1.63	166±1.24	3.6 ±0.1	3.7 ±0.1	224±1.82
13	Glutamic acid	1790±7.36	1891±6.23	1884±3.68	43.2 ±0.8	41.7 ±1.1	2829±19.34
14	Glycine	447±4.96	571±2.36	586±1.25	11.2 ±0.2	10.2 ±0.3	642±4.42
15	Proline	545±4.08	525±4.08	562±2.05	10.7 ±0.5	11.3 ±0.5	560±3.91
16	Serine	345±4.08	363±2.16	357±2.07	6.9 ±0.4	5.5 ±0.2	493±4.11
17	Tyrosine	314±4.32	328±8.83	332±2.05	3.0 ±0.2	4.0 ±0.3	411±2.95

The data were reported as means ±standard deviation, [a] n = 5 and [b] n=3

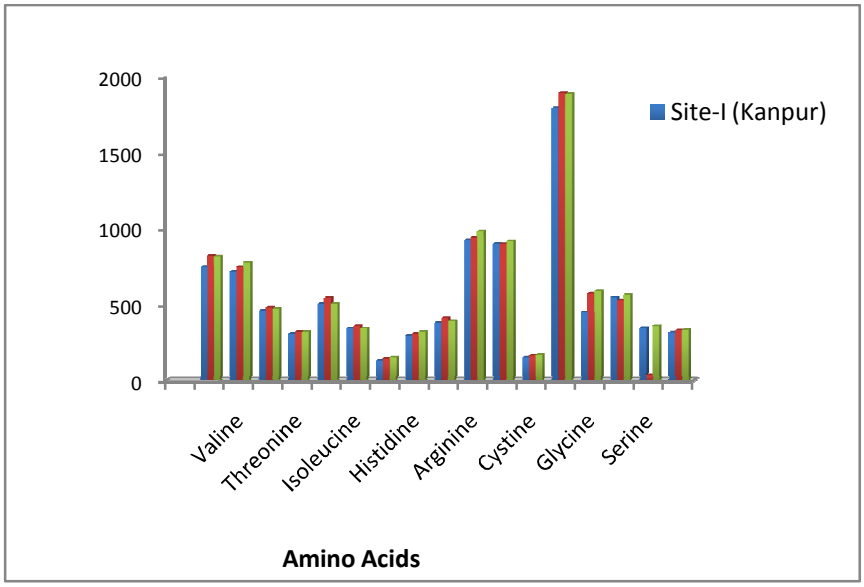

Figure 3 Essential and Nonessential Amino Acid in *Nigella sativa L.* seeds

CONCLUSION

The result obtained in this study showed that *Nigella sativa L.* seeds cultivated in Eastern Uttar Pradesh (India) are nutritious food that provide sufficient amount of nutrients, protein and vitamins needed for normal body function, maintenance and reproduction. The concentration ofsome of the trace metals, vitamins and essential amino acids were differed by the country of origin of *Nigella sativa L.* seeds. These differences could be explained by local growing conditions such as soil type and water. Results concluded from the study indicate that the *Nigella sativa L.* seeds can serve as a good nutritional source in combating malnutrition.

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