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Biochemical studies and anti-nutritional factors of lentil (*Lens culinaris medik*) varieties

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Abstract
The present research work entitled "Biochemical studies and anti-nutritional factors of lentil (*Lens culinaris Medik*) varieties" was conducted during *Rabi* season 2016-17 at the Agronomy research farm and laboratory of Agriculture Biochemistry Narendra Deva University of Agriculture & Technology, Kumarganj Faizabad (UP). Was adopted with three replications. Following lentil varieties were grown with proper agronomic practices and the seeds of ten varieties of Lentil namely NDL-1 (C) HUL-57 K-75 DPL-15 NDL-2 IPL-325 VL-148 NDL-22 PL-192 NDL-15 were undertaken to lentil varieties with successive were executed in Completely Randomized Design (CRD) was adopted with three replications. Following lentil varieties were grown with proper agronomic practices and the seeds of ten varieties were collected after harvesting and use for analysis of biochemical Parameters. viz Total chlorophyll content peroxidase enzyme). Were catalase activity. Tannin content and Phytic acid content. The data obtained in the experiment showed the highest total chlorophyll content at 30, 60, 90 and 120 days intervals in lentil varieties was recorded in the range of 0.40 to 0.48 mg/g, 0.35 to 0.45 mg/g, 0.26 to 0.40 mg/g and 0.10 to 0.19 mg/g. The data pertaining to the activity of catalase enzymes at 30, 60, 90 and 120 days in leaves of lentil varieties was found in the range of 36.07 to 39.87 Unit/g FW, 56.29 to 59.77 Unit/g FW, 67.97 to 70.81 Unit/g FW and 35.34 to 45.81 Unit/g FW NDL-1 variety. The activity of peroxidase enzymes at 30, 60, 90 and 120 days in leaves of lentil varieties was obtained in the range of 67.14 to 71.82 Unit/g FW, 87.27 to 91.92 Unit/g FW, 106.15 to 109.32 Unit/g FW and 78.54 to 81.34 Unit/g FW All the varieties were found significant. Highest tannin content was reported in the varieties IPL-325 (0.97 mg/g).and Maximum phytic acid content was recorded in the varieties IPL-325 (2.13 mg/g).

Keywords: Total chlorophyll content peroxidase enzyme). Were catalase activity, tannin content and Phytic acid content

Introduction
Lentil (*Lens culinaris medic.*) is one of the important and most nutritious *rabi* pulse. It has the potential to cover the risk of rain fed farming. It is also used as a cover crop to check the soil erosion in problem areas. The plants are ploughed back into the soil as green manure also. It derives the name *Lens* from the lens shaped seeds.

Lentil is known for their high nutrient content and health benefits in humans. It is also a great source of carbohydrates and high in fibre and important minerals such as iron and zinc. Lentil also contains essential amino acids isoleucine and lysine, making them a cheap source of protein in developing countries. The lentil plant, (*Lens culinaris medik)*, is a member of the Fabaceae (legume) family. Which is one of the major rabi pulse crop grown in India. To meet the demand for pulses, India has been importing a large quantity of pulses in recent years. In the global context, India is the largest producer of lentil. Which is grown under rain fed and unirrigated conditions, it adds to soil fertility. In India, lentil is mostly grown as a post rainy season crop under receding soil moisture conditions during the winter season. Lentil straw/husk is an important source of feed for animals feeding.

Lentil provide a variety of essential nutrients, containing high levels of protein (20%–30%), minerals (2%–5%), vitamins (folates), and prebiotic carbohydrates. Because of their numerous health benefits, high yield, and nitrogen benefit in food systems, lentil is a useful crop for micronutrient bio fortification efforts. Lentil is considered to be a good source of storage proteins, which are usually consumed by the germ during seed germination. In addition to providing essential and non-essential amino acids and carbon skeletons for the metabolic needs of the human body, lentil is source for some storage proteins that are described as biologically active proteins. Total carbohydrates represent the major component of lentil seeds with starches occupying most of the carbohydrate mass. Furthermore, lentil is a valuable source of total dietary fibres, with insoluble dietary fibre of approximately 93–99.7 %. The total alfa-galactosides or raffinose family oligosaccharides account for 53.0 % of the total sugars and oligosaccharides content in lentils. In these oligosaccharides, stachyose represents

the major oligosaccharide, followed by ciceritol and raffinose. The functional significance of these carbohydrates arises from their ability to work as selective promoters for the growth of beneficial gut microbes.

Ryan and colleagues found that lentil seeds contained a total fat of about 1.4 g/100 g, distributed unevenly over the fatty fractions namely: saturated fatty acids (SFA), 16.7 %; monounsaturated fatty acids (MUFA), 23.7 % and polyunsaturated fatty acids (PUFA), 58.8 %. Lentil world production increased from 3.78 million tonnes (Mt) in 2007 to reach 4.4 million tons in 2011, reflecting its nutritional significance. Lentil seeds contain high protein content, and considered the third-highest level of protein of any legume or nut, after soybeans and hemp. Seed protein content ranges from 22% to 34.6%. It also has high levels of carbohydrates (55%–59%) and elevated levels of micronutrients and vitamins.

Lentil contain a number of bioactive substances including enzyme inhibitors, lectins, phytates, oligosaccharides, and phenolic compounds that play metabolic roles in humans or animals that frequently consume these foods. These effects may be regarded as positive, negative, or both (Champ, 2002) [3]. Some of these substances have been considered as antinutritional factors due to their effect on diet quality. Enzyme inhibitors and lectins can reduce protein digestibility and nutrient absorption, respectively, but both have little effect after cooking (Lajolo & Genovese, 2002) [10]. Phytic acid can diminish mineral bioavailability.

Materials and Methods

The experiment was conducted during *Rabi* season 2016-17 at the Agronomy Research Farm and laboratory of Agriculture Biochemistry Narendra Deva University of Agriculture & Technology, Kumarganj Faizabad (UP). The biochemical parameters were as Total chlorophyll content the method as described by Arnon. (1949) [2] peroxidase enzyme was determined by method given by McCune and Galston (1959) [11]. The catalase activity was determined by the method of Sinha (1972) [16]. The tannin content in lentil was determined by method as given by Ranganna (1986) [14]. Phytic acid content in the lentil has been analyzed by the method of Wheeler and Ferrel (1971). The statistical analysis of the data obtained was carried out by the method as suggested by Gomez and Gomez (1984) [7].

Results and discussion

Chlorophyll content at various intervals was reported in decreasing pattern. The total chlorophyll content at 30, 60, 90 and 120 days intervals in lentil varieties was recorded in the range of 0.40 to 0.48 mg/g, 0.35 to 0.45 mg/g, 0.26 to 0.40 mg/g and 0.10 to 0.19 mg/g. The chlorophyll is an essential component of photosynthesis which occurs in the chloroplast and found in all green parts of the plant. They are bound loosely to protein but are extracted in organic solvent such as acetone or ether. Chemically each chlorophyll molecule contains prophoyrin (tetra pyrrole nucleus) with a chelated Mg atom at the centre and a long chain hydrocarbon in side chain attached to the carboxyl group. Similar range of total chlorophyll was also reported by Chandra *et al*. (2016) [4]. The highest chlorophyll content in crop at 30, 60, 90 and 120 days might be due to the proper temperature condition prevailing during its growth whereas least chlorophyll content in crop might be due to low temperature. Nitrogen content significantly influenced the chlorophyll content showing at 30, 60, 90 and 120 days as reported by Chandra *et al* (2016) [4]. Leaf chlorophyll content and chlorophyll a and b ratio were also recorded in decreasing pattern by Talukdar and Talukdar (2013) [17].

Table 1: Total chlorophyll content (mg/g) at 30, 60, 90 and 120 days in leaves of lentil varieties.

S. No.	Varieties	30 days	60 days	90 days	120 days
1.	NDL-1 (C)	0.48	0.45	0.40	0.19
2.	HUL-57	0.47	0.42	0.37	0.17
3.	K-75	0.47	0.43	0.38	0.18
4.	DPL-15	0.44	0.43	0.35	0.13
5.	NDL-2	0.42	0.39	0.30	0.15
6.	IPL-325	0.40	0.35	0.26	0.10
7.	VL-148	0.43	0.40	0.31	0.13
8.	NDL-22	0.44	0.41	0.32	0.14
9.	PL-192	0.42	0.36	0.28	0.11
10.	NDL-15	0.45	0.44	0.37	0.16
	SEM ±	0.010	0.014	0.015	0.013
	CD at 5%	0.029	0.042	0.044	0.038

The activity of catalase enzyme was found in increasing pattern up to 90 day followed by decrease at 120 days. The data pertaining to the activity of catalase enzymes at 30, 60, 90 and 120 days in leaves of lentil varieties was found in the range of 36.07 to 39.87 Unit/g FW, 56.29 to 59.77 Unit/g FW, 67.97 to 70.81 Unit/g FW and 35.34 to 45.81 Unit/g FW. Imani *et al.* (2013) observed significant correlation regarding activity catalase enzyme and noticed that the activity rate is increases at reproductive stage. The initial increases in the activity of this enzyme may be caused by excessive accumulation of reactive oxygen species as reported by Jiang and Hung, (2001) [9]. Shao *et al* (2005) showed that reaching maturity and ageing, decreased the activity of peroxidase and catalase enzymes.

Table 2: The activity of catalase enzyme (Unit/g FW) at 30, 60, 90 and 120 days in leaves of lentil varieties.

S. No.	Varieties	30 days	60 days	90 days	120 days
1.	NDL-1 (C)	39.87	59.77	70.81	45.81
2.	HUL-57	38.76	58.68	69.25	45.09
3.	K-75	39.12	59.03	69.94	43.04
4.	DPL-15	38.14	58.21	69.35	44.37
5.	NDL-2	37.16	57.25	68.34	43.46
6.	IPL-325	36.07	56.29	67.97	35.34
7.	VL-148	37.42	57.59	68.76	43.91
8.	NDL-22	37.86	57.74	69.14	44.09
9.	PL-192	36.75	56.75	68.12	43.24
10.	NDL-15	38.34	58.45	69.03	44.83
	SEM ±	0.0201	0.0072	0.0201	0.0145
	CD at 5%	0.058	0.021	0.058	0.042

The activity of peroxidase enzyme was found in increasing pattern up to 90 day followed by decrease at 120 days. The activity of peroxidase enzymes at 30, 60, 90 and 120 days in leaves of lentil varieties was obtained in the range of 67.14 to 71.82 Unit/g FW, 87.27 to 91.92 Unit/g FW, 106.15 to 109.32 Unit/g FW and 78.54 to 81.34 Unit/g FW. Peroxidase is one of the enzymes involved in oxidation of phenolic compounds and converts them into complex condensation products brown in colour. In plants, this enzyme was involved in the resistance against infection and in the synthesis of plant constituents. This enzyme also acts as oxygen scavenger in photosynthetic tissues. The enzyme catalyses the oxidation of monophenols (Tyrosin-crisol and O-phenols) and diphenols (Pyrogallol, catechol). Increasing activity of the enzyme at different intervals may be due to the phenolic compounds which are present in lentil crop. The increasing activity on this enzyme in potato cultivar is also reported by Ezkil *et al.* (2007) [5] may be due to oxidation of tyrosine: 3, 4, dihydroxyphenyl alanine which is finally converted in dopa, quinone and dopamine by catalysing this enzyme.

Table 3: The activity of peroxidase enzyme (Unit/g FW) at 30, 60, 90 and 120 days in leaves of lentil varieties.

S. No.	Varieties	30 days	60 days	90 days	120 days
1.	NDL-1 (C)	71.82	91.92	109.32	81.34
2.	HUL-57	70.26	90.36	108.75	80.85
3.	K-75	70.96	90.89	109.04	81.04
4.	DPL-15	70.41	90.24	108.23	80.13
5.	NDL-2	69.41	89.32	107.11	79.24
6.	IPL-325	67.14	87.27	106.15	78.54
7.	VL-148	69.80	89.74	107.25	79.75
8.	NDL-22	70.36	90.45	107.84	80.02
9.	PL-192	68.03	88.13	106.17	79.06
10.	NDL-15	68.89	88.81	108.62	80.46
	SEM ±	0.0037	0.0136	0.0271	0.0148
	CD at 5%	0.011	0.039	0.079	0.043

Anti-nutritional factors in seeds of lentil varieties

The tannin content was noticed in the range of 0.54 to 0.97(mg/g). Muehlbauer and Sarker (2011) [12] developed some of tannin free varieties and they also noticed that lentil varieties contain poly phenolic compound that slowly oxidised when expose to the air and turns into brown colour. Tannin is polyphenolic materials which are able to precipitate protein from solution. They contain O-dihydroxy phenol groups which allow them to from hydrogen bonds and hydrophobic bonds with protein. The typical structure of hydrolysable tannin, the gallo tannin and ellagi tannin is characterized by control polyhydroxy moiety usually β.D. glucopyranose. The important gallotannin component in pentagelloyl glucose which react further with gallate molecule to from meta depiside bonds. High tannin content in this crop causes resistance to birds. Tannin are hydrolysable compound which consists of simple phenolics such as gallic acid condensed with glucose molecule. The condensed tannin accumulate in vacuoles and have much higher molecular weight than gallotannin, the monomeric flavonoid are involved in proanthocyanidins biosynthesis and form polymerized flavonols such as catchin and epicatchin.

Table 4: Tannin (mg/g) and phytic acid content (mg/g) in lentil varieties.

S. No.	Varieties	Tannin content (mg/g)	Phytic acid content (mg/g)
1.	NDL-1 (C)	0.54	0.98
2.	HUL-57	0.61	1.33
3.	K-75	0.58	1.12
4.	DPL-15	0.68	1.61
5.	NDL-2	0.82	1.97
6.	IPL-325	0.97	2.13
7.	VL-148	0.79	1.84
8.	NDL-22	0.72	1.72
9.	PL-192	0.91	2.07
10.	NDL-15	0.63	1.52
	SEM ±	0.0108	0.0183
	CD at 5%	0.03	0.05

Data pertaining to the phytic acid content in different lentil varieties was observed in the range of 0.98 to 2.13(mg/g). Qayyum *et al.*, (2012) [13] found similar range of phytic acid in lentil crop and found that heat treatment dissociates phytates complex especially in this pulse. Phytic acid is an important parameter of legume crop seeds which serve as store of phosphate for seedling, but its phosphate group carries negative charges which bind with calcium, iron and zinc ions. This reduces the availability of these minerals to human being and can result in deficiency symptom even when the diet appears adequate. The phosphate group remove from phytic acid through the action of enzyme phytase which is produced by germinating seeds, fungi and microorganism, thus phytate in the diet of human being is broken down being and phosphate is made available.

Conclusions

On the basis of overall observations, it can be concluded that Lowest Tannin content (0.54 mg/g), Phytic acid (0.98 mg/g) were reported in NDL-1. A decreasing trend was reported regarding chlorophyll content up to 30, 60, 90 and 120 days. The activity of anti-oxidant enzymes namely catalase and peroxidase were found in increasing pattern up to 90 day followed by decrease at 120 days.

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