The Pharma Innovation Journal 2022; 11(11): 1714-1718

www.ThePharmaJournal.com

The Pharma Innovation

ISSN(E): 2277-7695
ISSN(P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(11): 1714-1718

www.thepharmajournal.com
Received: 21-09-2022
Accepted: 24-10-2022

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Estimation of the effect of different nitrogen levels and different spacing on biochemical parameter on Quinoa (*Chenopodium quinoa* Willd.)

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Abstract
Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal traditionally consumed by Andean cultures that is attracting attention worldwide as a functional food. Because of its tolerance to extreme environmental conditions and its nutritional and biological properties, quinoa has been defined as 'one of the grains of the 21st century'. In addition to its high content in protein, lipids, fiber, vitamins, and minerals, and its excellent balance of essential amino acids, quinoa has been found to contain numerous phytochemicals including saponins, phytosterols, phytoecdysteroids, phenolics and bioactive peptides. These compounds may exert beneficial effects on metabolic, cardiovascular, and gastrointestinal health. This paper summarizes the effect of different nitrogen levels and different spacing on biochemical parameter.

Keywords: Pseudocereal, environmental, nutritional, saponins, nitrogen etc

1. Introduction
Quinoa (*Chenopodium quinoa* Willd.), a seed crop in the amaranthaceae family, has been farmed in the Andean region for thousands of years for its nourishing grain and leaves (Pearsall, 1992) [8]. Quinoa (*Chenopodium quinoa*) is a dicotyledonous plant endemic to South America's Andean highlands. Quinoa seeds are round and flat, with a diameter of 1.4-1.6 mm (Abugoch, 2009) [1]. They are made up of a huge core perisperm and an embryo on the periphery. The endosperm is only present as a cap around the radicle tip in the micro Pilar area of the seed (Prego *et al*., 1998) [11]. Quinoa grains are used in a variety of ways, in clod ing cooking, baking, animal feed, green fodder, and pellets; modified foods such breakfast cereals, pasta, and pastries; industrial uses for carbohydrate, protein, and saponin; and as a cover crop.
Because the crop, which is a pseudo-cereal, includes gluten-free, high-quality protein, it can play a significant role in celiac disease sufferers' diets (Kuhn *et al*., 1996; Dowidar and Kamel, 2011) [7, 5]. It contains lysine, a limiting amino acid in cereal; twice that of wheat; differs from other crops amino acid composition, is more attractive and highly balanced similar to that of casein; contains lysine, a limiting amino acid in cereal(Rathore S., *et al.*, 2019) [9]. Important amino acids, such as methionine, threonine, and lysine, are abundant in quinoa seed protein, which are absent in most cereal grains (Bhargava *et al*., 2007; Comai *et al*., 2007) [3, 4]. The Food and Agriculture Organization of the United Nations (FAO) has designated 2013 as Quinoa Year (Anonymus, 2013) [2].

2. Materials and method
2.1 Protein Content
Proteins are polymers of amino acids. Twenty different types of amino acids occur naturally in proteins. Proteins differ from each other according to the type, number and sequence of amino acids that make up the polypeptide backbone. As a result they have different molecular structures, nutritional attributes and physiochemical properties. Proteins are important constituents of foods for a number of different reasons. They are a major source of energy, as well as containing essential amino-acids, such as lysine, tryptophan, methionine, leucine, isoleucine and valine, which are essential to human health, but which the body cannot synthesize. Protein content of quinoa was calculated by kjeldahl method which are as follows:

2.1.1 Kjeldahl method

The Kjeldahl method was developed in 1883 by a brewer called Johann Kjeldahl. Plant material is digested with a strong acid so that it releases nitrogen which can be determined by a suitable titration technique. The amount of protein present is then calculated from the nitrogen concentration of the plant material. The same basic approach is still used today, although a number of improvements have been made to speed up the process and to obtain more accurate measurements (Wang, 2021) [10]. It is usually considered to be the standard method of determining protein concentration. Because, the Kjeldahl method does not measure the protein content directly, a *conversion factor (F)* is needed to convert the measured nitrogen concentration to a protein concentration. A conversion factor of 6.25(equivalent to 0.16 g nitrogen per gram of protein) is used for many applications, however, this is only an average value.

2.2 Leaf Nitrogen Content

The dry leaves were then ground separately and passed through a sieve to get finely powdered samples. The powdered samples were then fed into a Thermo – Fisher Scientific Flash Smart Elemental Analyzer (CHNS instrument) to obtain the nitrogen content in each leaf sample. A total of 272 leaf samples were analyzed using the CHNS instrument. The CHNS analyzer works on the Dumas method in which flash combustion is done for instantaneous oxidation of the sample. Later, a chromatographic column and thermal conductivity detector were used to separate and detect the combustion products (Fadeeva 2008) [6]**.** The CHNS analyzer was calibrated using k-factor analysis of 2.5-Bis (5-*tert*-butyl-benzoxazol-2-yl) thiophene (also known as BBOT). Leaf powder samples in a quantity of 3-4 mg was used for the analysis. Each leaf sample was replicated twice, and every 50th sample was replicated thrice in the CHNS analyzer to check the consistency of the results. Average values of these replications were assigned as ground-truth leaf nitrogen content. The CHNS-based LNC was considered actual LNC.

2.3 Saponification Value

This method is used to determine the total acid content, both free and combined, of tall oil. (Acid number only measures the free acid). The combined acids are primarily esters formed by reaction with the neutral components present in the original tall oil. The saponification value is therefore a measure of tall oil quality. It is determined by measuring the alkali required to saponify the combined acids and neutralize the free acids.

Apparatus

1. Erlenmeyer flask, 300-mL with S/T 24/40 neck and reflux condenser.
2. Pipet, 25-mL.
3. Automatic titrator (optional).

Reagents

1. Potassium hydroxide, 0.5 N solution in 3A etjyl alcohol, standardized to ±0.005
2. Hydrochloric acid, 0.5 N solution, standardized to ±0.005.
3. Phenolphthalein indicator, 1%(visual titration only)

Procedure

1. Weigh 2 g of sample, to the nearest 0.01 g, into a 300-niL Erlenmeyer flask.
2. Using a pipet, add 25 n1L of 0.5 N ethanolic potassium hydroxide.
3. Reflux for 60 minutes.
4. Titrate between 60 and 70ºC with 0.5 N hydrochloric acid using phenolphthalein
5. Indicator or an automatic titrator.
6. Run a blank in the same manner.

Calculation

$$Saponification\ Value = \frac{(A-B)\times N\times 56.1}{W}$$

Where,
A= H_2SO, for blank, mL
B= H_ZSO, for sample, mL
W= weight of sample (dry basis), g
N= normality H_2SO_4 solution
56.1= equivalent weight of potassium hydroxide

Precision statement

Based on an ASTM round-robin study, the within laboratory (repeatability) standard deviation for this test is 0.8 and the between laboratory (reproducibility) standard deviation for this test is 1.4.

3. Result and Discussion

The mean data on biochemical parameters viz. leaf nitrogen content (%), Protein content (%) and Saponification value affected by different treatments was summarized and presented in Table 1.

3.1 Protein content (%)

The mean data of pooled experimental observation regarding protein content showed significant effect under spacing as shown in figure 1. However, maximum protein content (%) was observed with S2 - 45 cm (16.25%) and lowest with S1 - 30 cm (13.24%). Nitrogen scheduling depicted significant effect on protein content as shown in figure 2. Maximum protein content was analyzed with N3 - 120 kg(15.32%) which was statistically at par with N2 - 80 kg(14.70%) but significantly superior over N1 - 40 kg(14.98%).

3.2 Leaf nitrogen content (%)

The mean data regarding leaf nitrogen content showed significant effect under spacing as shown in figure 3. However, maximum leaf nitrogen content (%) was observed with S2 - 45 cm (1.72%) and lowest with S1 - 30 cm (1.64%). Nitrogen scheduling showed significant effect on leaf nitrogen content as shown in figure 4. Maximum leaf nitrogen content was analyzed with N3 - 120 kg(1.77%) which was statistically at par with N2 - 80 kg(1.61%) but significantly superior over N1 - 40 kg(1.60%).

3.3 Saponification value

An appraisal on mean data regarding saponification value revealed significant effect under spacing as shown in figure 5. However, maximum saponification value was noted with S2 - 45 cm (192.22) and lowest with S1 - 30 cm (185.95).

Nitrogen scheduling depicted significant effect on saponification value as shown in figure 6. Maximum saponification value was analyzed with N3 - 120 kg(189.78) which was statistically on par with N1 - 40 kg(188.82) but significantly superior over N2 - 80 kg(188.57). Hence, treatment N3 and N1 was not significantly different.

Table 1: Protein Content, Leaf Nitrogen Content and Saponification Value

S. No.	Factors	Protein Content		Pooled	Leaf Nitrogen Content		Pooled	Saponification Value		Pooled
		Rabi Season 2019-20	Rabi Season 2020-21		Rabi Season 2019-20	Rabi Season 2020-21		Rabi Season 2019-20	Rabi Season 2020-21	
A	**Spacing**									
	S1 - 30 cm	13.30	13.18	13.24	1.48	1.77	1.62	184.73	187.16	185.95
	S2 - 45 cm	16.19	16.31	16.25	1.55	1.89	1.72	191.93	192.50	192.22
	S3 - 60 cm	15.69	15.31	15.50	1.44	1.83	1.64	188.45	189.57	189.01
B	**Nitrogen Scheduling**									
	N1 - 40 Kg.	15.12	14.83	14.98	1.44	1.76	1.60	188.21	189.43	188.82
	N2 - 80 Kg.	14.80	14.60	14.70	1.42	1.79	1.61	187.38	189.76	188.57
	N3 – 120 Kg.	15.25	15.38	15.32	1.61	1.93	1.77	189.52	190.05	189.78
C	S.Em±	0.297	0.282	0.200	0.080	0.084	0.079	0.792	0.563	0.528
D	C.D. at 0.05	0.866	0.822	0.583	0.233	0.245	0.232	2.310	1.644	1.540
E	CV (%)	3.94	3.77	2.66	10.74	9.17	9.56	0.84	0.59	0.56

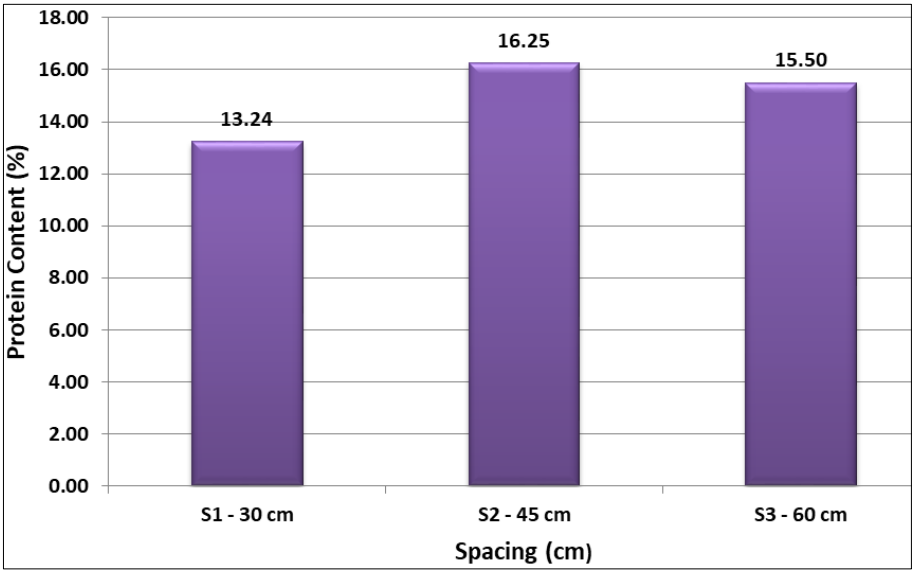

Fig 1: Variation of Protein content (%) with respect to Spacing

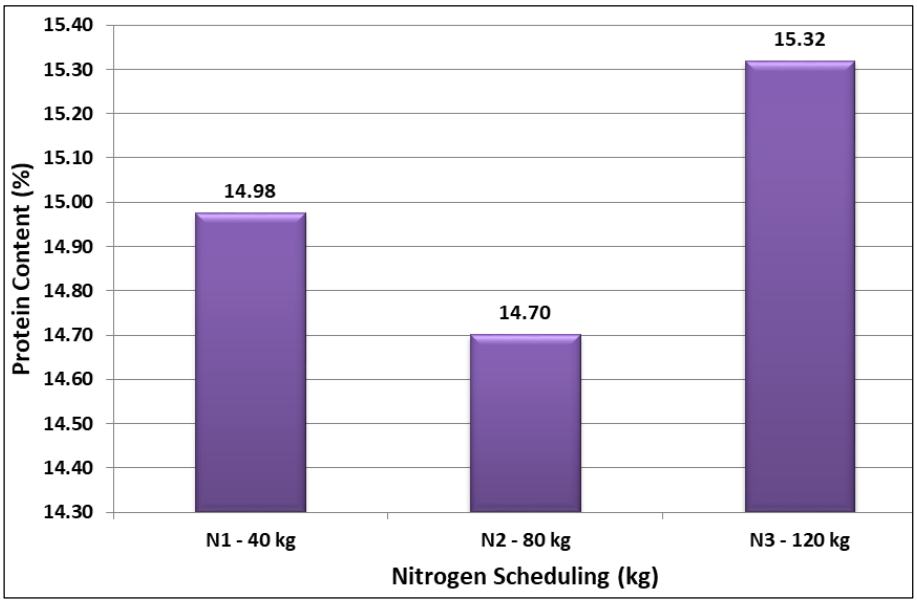

Fig 2: Variation of Protein content (%) with respect to Nitrogen Scheduling

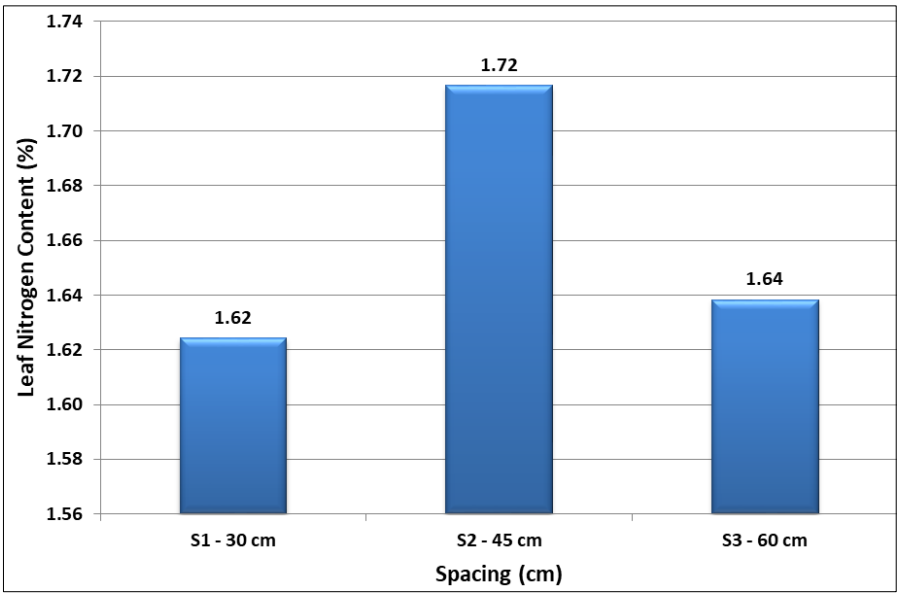

Figure 3: Variation of Leaf Area Content (%) with respect to spacing

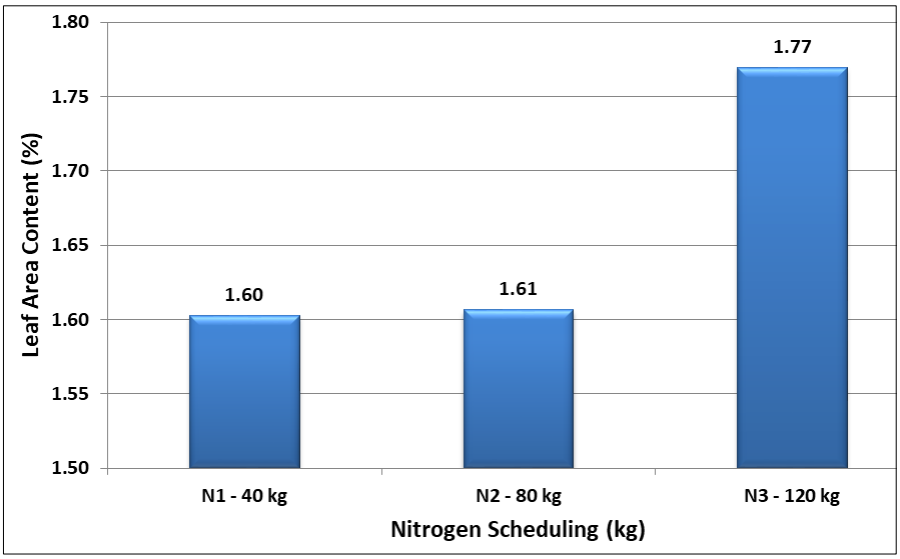

Fig 4: Variation of Leaf Area Content (%) with respect to Nitrogen Scheduling

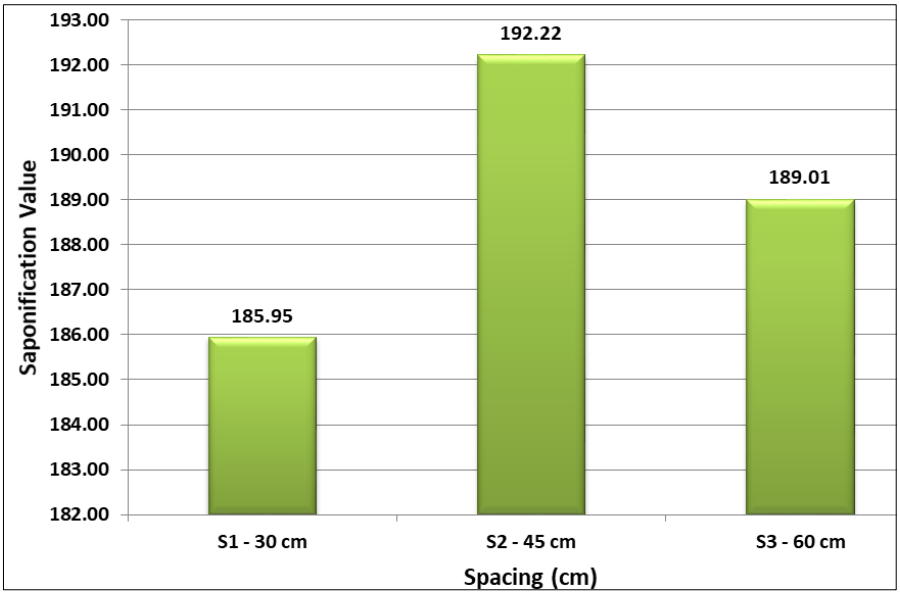

Fig 5: Variation of Saponification value with respect to spacing

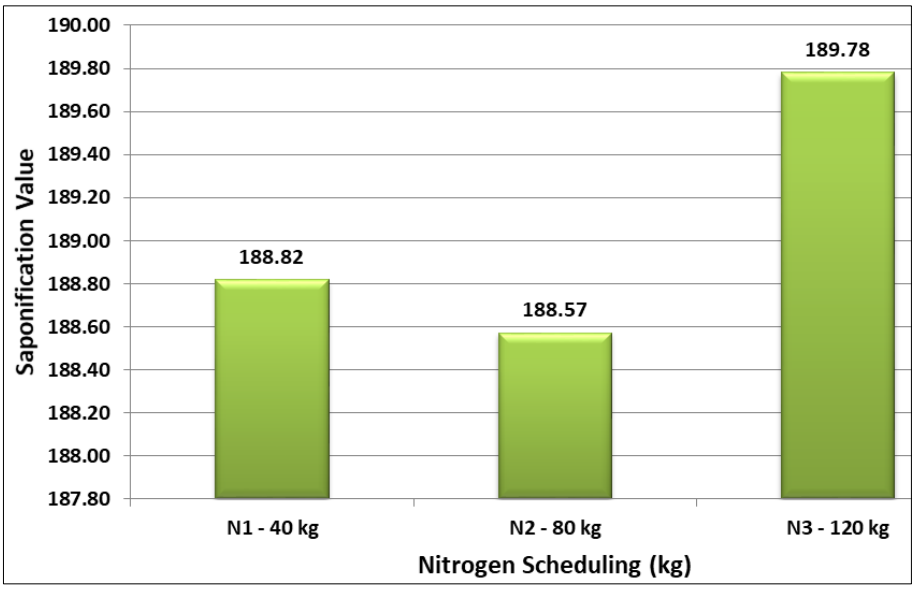

Fig 6: Variation of Saponification value with respect to Nitrogen Scheduling

4. Conclusion

Quinoa is a pseudocereal with an important tradition and notable environmental tolerance, in addition to its high nutritional value. It has been recently reported that one serving of quinoa (about 40 g) meets a significant part of daily recommendations (RDA) for essential nutrients, mainly vitamins, minerals and essential amino acids. Treatment S2-45 cm had the highest protein content, leaf nitrogen content and saponification value. Nitrogen scheduling has a large impact on biochemical parameters. N3 - 120 kg had the highest protein content, leaf nitrogen content and saponification value.

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