

Proximate and Biochemical Analysis of Water Chestnut (*Trapa natans* Var. *bispinosa* Roxb) kernel as affected by various inorganic and organic sources

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ABSTRACT: The water chestnut plays a significant role in wetland zones in India, however artificial fertilizers that are harmful to human health are employed to keep up with the fast-growing production rate. The present investigation aimed to know the impact of various organic and inorganic fertilizers on the proximate value and biochemical parameters of water chestnuts. The water chestnut was treated with half of the RDF through (Urea + DAP) (T₁), one-fourth of the RDF through Urea + DAP (T₂), and Nano-Urea @ 4.0%. T₅ Jivamrut (10%) 100% RDF. The treatment T₄ i.e. nano urea (4%) significantly enhanced the value of most of the parameters. The highest N (2.68%), C (53.66%), H (8.49%), C/H ratio (6.31), protein (16.75%), S (0.29%) were most significant in T₅ (@ Jivamrut 10%) and amylose (16.24%) observed in T₁. For biochemical studies under different nutritional regimes where T₃ (¼ RDF–Urea + DAP) had the greatest moisture (19.13%) content, T₅ (@ Jivamrut 10%) had the highest TSS (3.80°brix), and T₄ (Nano-Urea @ 4.0%) had the highest acidity (0.13%).

Keywords: Minerals, Proximate analysis, Biochemical analysis, Protein, Carbohydrate.

INTRODUCTION

The annual herbaceous aquatic free-floating plant known as water chestnut (*Trapa natans* var. *bispinosa* Roxb). In India, it is referred to as Singhara locally and water chestnut globally. (Li *et al.*, 2021 and Gond *et al.*, 2018) Commercial water chestnut cultivation occurs in tropical freshwater ponds, lakes, rivers, and wetlands. The water chestnut is mostly grown around the world in places like China, Africa, India, *etc.* According to India's wetland environment, it is the most significant aquatic fruit (Walde *et al.*, 2016). *Trapa bispinosa*, one of the significant fruit-bearing genera, is frequently seen in lakes, ponds, tanks, pools, and many other water reservoirs. The cultivated species among the many *Trapa* species include *natans*, which is a major food crop in temperate countries, and *T. quadrispinosa*, which has two large and two little-sized spines (Mohinuddin *et al.*, 2020). The northeastern and Eastern regions of the country are home to the greatest instances of *T. bispinosa* among these three domesticated species (Yang *et al.*, 2020). The crop can be readily farmed on a big scale with great profitability because it does not compete with land crops. The crop may also be grown in water basins with a standing water level of 60 to 180 cm and fish culture tanks (Singh *et al.*, 2015). Warm humid weather is preferred for the cultivation of water chestnuts. Frost won't let it stand (Taher *et al.*, 2023). According to (Gond *et al.*, 2018) *Trapa* requires high

summer temperatures for successful cultivation. The climatic conditions of the tropical and subtropical zones, where this crop is produced economically, for germination 15-20°C temperature is required (Nie *et al.*, 2021). The fruit has a carbohydrate content of roughly 80%, a protein content of 5%, and a substantial number of vitamins (Alam *et al.*, 2021). The delectable kernel has important minerals, proteins, and carbohydrates. Additionally, it contains a lot of vitamins B₁, B₂, B₅, B₆, E, A, and C (Singh *et al.*, 2015). According to the fruit of the water, chestnut is rich in a variety of nutrients, including minerals (P, Ca, K, Fe, and Zn), vitamins (Vitamin B, C, and D), citric acid, lipids, crude fiber, and proteins. Numerous phytonutrients, including catechins and epicatechins antioxidants and "puchin," a substance resembling penicillin, are present in water chestnut. Along with the singhara, the mixture also includes vanillin, hydrocinnamic acid, gallic acid, and p-coumaric acid (Taher *et al.*, 2023). Water chestnut is cultivated by the local farmers on the wetland site over an approximate area of 250 Ha. However, in a survey conducted in 2021-22 (Srivastava, 2022; Mishra 2022). it was found that the local farmers are making indiscriminate applications of chemicals in the form of fertilizers, pesticides, and insecticides with the sole objective of high yield (Garg *et al.*, 2020). Chemical fertilizers like Urea and Diammonium phosphate are applied at three times the recommended doses. To

overcome this the farmers, treat the fruit with a bleaching agent to improve its appearance so that the market acceptability is high. It is hypothesized that varying doses of chemical fertilizers, Nano urea, and Jivamrut could improve the performance of the crop without affecting its overall yield.

MATERIAL AND METHODS

A. Sample Preparation

Fruit kernels were randomly selected from farmer fields, and then a sharp knife was used to extract the kernels from the skin. After two days of sun drying, the kernels were oven-dried at 70°C until they reached a consistent weight. 200 samples of each treated treatment were prepared after the kernels had been granted with the aid of a grander and power sieved through a 500-micrometer diameter sieve after 24 hours.

B. Treatment details

Three replications and six treatments are included in experiments conducted using a completely randomized design. In this experiment, six treatments were carried out: T₁ Control, T₂ ½ RDF (Urea + DAP), T₃ ½ RDF (Urea + DAP) 19, T₄ Nano-Urea @ 4.0%, T₅ Jivamrut @ 10%, and T₆ RDF.

C. Proximate Analysis

Nitrogen %. Nitrogen was determined using the micro-Kjeldahl method. About 2 g of dried sample was transferred into a digestion tube by adding 2 tablets of catalyst and 20 mL of sulfuric acid to digestion in 30 min using a Kjeldahl digester (Tecator Kjeltex System, Germany) at a minimum temperature of 400°C. After that, 50 mL of distilled water was added for distillation using Kjeldahl distillation. Then, the sample was titrated with hydrochloric acid (0.20 N) to calculate the amount of HCL present in the NaOH solution (40%). The boric acid solution (4%) was used for the catalyst reagent. The percentages of nitrogen were converted to protein by multiplying by 6.25.

Carbon %. Carbon determination: Weigh a portion of the dried fruit sample (usually around 0.5-1.0 grams). Carbon analysis can be done using techniques like dry combustion (usually around 900-1000°C) or using a carbon analyzer. (Lussier *et al.*, 1994 and Gond *et al.*, 2018).

Hydrogen %. The same combustion process can be used to estimate the hydrogen content in the sample. In this step, the combustion products containing carbon dioxide are removed from the apparatus. The remaining residue, which contains water vapor, is subjected to further high-temperature heating (generally around 700-800°C) in the presence of a catalyst, such as copper or nickel. The water vapor in the residue is then converted into hydrogen gas (H₂), which can be captured and measured using suitable techniques like gas chromatography. (Singh *et al.*, 2014). Growth, photosynthesis and oxidative responses of *Solanum melongena* L. seedlings to cadmium stress: mechanism of toxicity amelioration by kinetin.

Sulphur %. A known quantity of water chestnut is burnt completely in a current of oxygen. Ash, thus

obtained, contains Sulphur of the water chestnut as sulfate which is extracted with diluted hydrochloric acid. The extract is treated with barium chloride solution to precipitate the sulfate as barium sulfate. (Chakraborty *et al.*, 2009).

C/N Ratio. The ratio of C/N is determined by the formula weight of total carbon divided by the weight of total nitrogen from the selected sample.

C/H Ratio. Once the carbon and hydrogen percentages are determined, the C-H ratio can be calculated by dividing the percentage of carbon by the percentage of hydrogen.

Protein %. The extracted soluble fraction from the Fibre bag system was examined for various food and paper wastes using the Lowry technique (Lowry *et al.*, 1951), calibrated on bovine serum albumin.

Carbohydrate %. Different fiber fractions were quantified as the particulate carbohydrate content of hemicellulose, cellulose, and lignin, as determined above by the sequential extraction using neutral and acid detergents, followed by strong acid extraction for the cellulose content. Total sugars were measured with the Anthrone reduction method (Gond *et al.*, 2018).

Amylose. The most frequent method used to determine amylose content is amylose-iodine colorimetry performed using a spectrophotometer. Liner amylose produces a deep blue complex when it reacts with iodine, which is measured by light absorption spectroscopy. (Tortoe *et al.*, 2017).

D. Biochemical Analysis

Moisture %. To calculate the moisture content in percent, the water chestnut kernel and peel are dried in a hot air oven to maintain an even weight. The weight loss is then measured. This technique is used to estimate the fruit's moisture content at 50°C. (Panda *et al.*, 2022).

TSS (°Brix). Total soluble solids of the fruit were estimated by placing a drop of fruit juice on the prism of the Hand refractometer (Model: Atago, Japan) and the reading against the light was recorded and expressed as °Brix (Meena and Asrey 2018).

Total Acidity (%). The titratable acidity of the kernel was estimated by titration method as described by Ranganna, 2000 and followed by (Meena and Asrey, 2018). For this, a filtered 10 g sample was titrated against 0.1 N NaOH by adding a drop of naphthalin until pink color.

E. Statistical analysis

The data recorded for evaluation of different treatments in tomato and cucumber was statistically analyzed using the standard procedure as suggested by (Panse and Sukhatme 1985) for analysis of variance of F (CBD) to test the significance.

RESULT

A. Proximate Analysis

The current examination's results (Table 1) indicated that the largest percentages of amylose (12.84%), protein (16.75%), carbs (84.04%), hydrogen (8.49%), sulfur (0.29%), carbon (53.66%), nitrogen (2.68%), and hydrogen/nucleus ratios (26.82) were found. Protein,

carbon, hydrogen, sulfur, nitrogen, C/H ratio, and sulfur were most significant in T₅ (@ Jivamrut 10%). T₃ (¼ RDF – Urea + DAP) had the highest C/N ratio, although T₁ (Control) had the largest percentages of carbs and amylose. However, T₃ (¼ RDF – Urea + DAP) had the lowest levels of protein, carbon, hydrogen, sulfur, C/H ratio, and nitrogen; T₅ (@ Jivamrut 10%) had the lowest C/N ratio; T₂ (½ RDF – Urea + DAP) had the lowest C/H ratio; T₆ (RDF) had the lowest levels of carbohydrates; and T₅ (@ Jivamrut 10%) had the lowest levels of amylose.

B. Biochemical Analysis

Standard measurements were made to estimate the biochemical analysis. Table 2 displays the kernel samples with the greatest levels of moisture (19.13%), total acidity (0.13%), and TSS (3.800brix). where T₃(¼ RDF – Urea + DAP) had the greatest moisture content, T₅ (@ Jivamrut 10%) had the highest TSS, and T₄ (Nano-Urea @ 4.0%) had the highest acidity percentage. In contrast, T₆ (RDF) had the lowest moisture content, and T₁ (control) had the lowest TSS and total acidity percentage.

DISCUSSION

The proximate analysis typically involves the determination of moisture, volatile matter, fixed carbon, and ash, and represents the most frequently used method for biofuel characterization (Thipkhumthod *et al.*, 2005; García *et al.*, 2012). The proximate analysis includes moisture, crude fat, crude protein, amylose, nitrogen, carbon, hydrogen, and total carbohydrates result was also reported by some earlier researchers (Adnan *et al.*, 2010) and (Hassan *et al.*, 2010). Fixed

carbon refers to carbon in its free state, not combined with other elements (UN, 2006).

The undefeated Moringa samples had a higher fat content of 38.67 %. The value was lower than the value (42%) reported by Ogunsina *et al.*, (2011) and higher than the value (30.36-35.20%) reported by Anwar *et al.*, (2021) for raw Moringa seed flour. This variation in crude fat content, according to crude fat values in kernels was high by different values as compared to values reported by other researchers: 1.77 (Ogungbenle and Atere, 2014) and 5.11±0.10% (Oladimeji and Bello, 2011) but lower than 47.00% (Ndabikunze *et al.*, 2006) in *P. curatellifolia* kernel.

The protein content in the pulp was significantly lower than in the corresponding kernels (p<0.05). *A. digitata* kernels contained on average 39.3% protein and *S. birrea* kernels 32.6%. For *A. digitata*, our results are by other results. The proximate composition of the kernel indicates it is highly nutritious as it contains high protein content, hence could supplement other protein sources such as beans, peas, and groundnuts especially in dry seasons and in arid regions.

The crude protein content of the kernel was found to be greater than that of leaves (Kubmarawa *et al.*, 2008), and flower, and fruit pulp crude fat values in kernels were high by different values as compared to values reported by other researchers: 1.77 (Ogungbenle and Atere 2014) (Ndabikunze *et al.*, 2006) as reported for studies conducted in Nigeria and Tanzania. The crude fat value of 46.05±0.47 for *P. curatellifolia* kernel was higher than 39.10% (Kumar *et al.*, 2013), but similar to 47.00±0.03 (Atasie *et al.*, 2011) for groundnuts (*Arachis hypogaea*).

Table 1: Proximate analysis of water chestnut (*Trapa natans* Var. *bispinosa* Roxb).

Kernel									
Treatments	N%	C%	H%	S%	C/N ratio	C/H ratio	Protein %	Carbohydrate %	Amylose (mg)
T ₁	1.67	39.77	6.353	0.196	23.86 04	6.2606	10.44	84.04	16.24
T ₂	1.66	39.68	6.364	0.227	23.8377	6.2354	10.38	78.74	14.84
T ₃	1.46	39.29	6.276	0.196	26.8295	6.2601	9.13	78.87	15.83
T ₄	2.07	44.17	7.083	0.243	21.3456	6.2365	12.94	74.01	14.28
T ₅	2.68	53.66	8.498	0.298	20.0413	6.314	16.75	78.43	12.84
T ₆	2.10	45.61	7.26	0.227	21.7068	6.282	13.13	73.72	13.13
CD	0.074	1.4	0.259	0.01	0.8	N/A	0.412	2.637	0.46
SEM	0.024	0.449	0.083	0.003	0.257	0.071	0.132	0.846	0.148

Note – T₁ Control, T₂ : ½ RDF (Urea + DAP), T₃ : ¼ RDF (Urea + DAP), T₄ : Nano-Urea @ 4.0% . , T₅:Jivamrut@ 10 % , T₆ : RDF

Table 2: Biochemical analysis of Water Chestnut (*Trapa natans* Var. *bispinosa* Roxb).

Kernel			
TREATMENT	MOISTURE %	TSS (°Brix)	Total Acidity %
T ₁ : Control	17.56	3.30	0.07
T ₂ : ½ RDF (Urea + DAP)	16.04	3.70	0.09
T ₃ : ¼ RDF (Urea + DAP)	19.13	3.40	0.08
T ₄ : Nano-Urea @ 4.0%	16.42	3.60	0.13
T ₅ : Jivamrut @ 10 %	17.53	3.80	0.08
T ₆ : RDF	15.13	3.50	0.10
CD	0.633	0.15	0.003
SEM	0.203	0.048	0.001

The Biochemical study was investigated in these studies under different nutritional regimes. According to hierarchical analysis based on biochemical parameters, the Moroccan walnut accessions were structured into four distinct groups independently of the mountain range type. Corroborating it, the results obtained by Kabiri *et al.*, (2018) by analyzing the same accessions using pomological and morphological traits. The total soluble solids of the kernel were recorded in all treatments from 3.30% to 3.80%. A similar report has been reported in apples and sweet peppers the biochemical composition of three aquatic macrophytes, on average, *T. bispinosa* contained the highest amounts of carbohydrate (71.55%) and the lowest amounts of protein (10.80%) on a dry weight basis (Alfasane *et al.*, 2009). Howard-Williams and Junk studied the nutritional values of 27 Amazonian macrophyte species and found 5.3-22.2% protein in the whole biomass on a dry weight basis. The highest was found in *Azolla microphylla* Kaulf. and the lowest in *Scleria secans* (L.) Urb. and *Rhynchospora gigantea* Link. the titratable acidity was 7.8% for *A. digitata*, 0.9% for *S. birrea*, and 1.7% for *V. infant* (Amarteifio *et al.*, 2009). Comparable data were 0.3% for mango pulp and 0.7% for orange juice (Okia *et al.*, 2011).

CONCLUSION

Water chestnut is a minor fruit crop of India it is a tremendous nutritive fruit among unadopted fruit crops. Present-time proximate and biochemical analysis is a modern and popular method to detect many nutrients in one attempt. In the present investigation, the proximate result was found superior in treatment T₅ (Jivamrut) and minimum T₁ (control). Biochemical analysis (kernel) superior result found in treatment T₅ (Jivamrut).

Conflict of interest. There is no conflict of interest and there is no ethical issue on animal behavior.

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