

Impact of different formulations of biofertilizer consortia (NPK & NPKZn) on proximate nutritional composition of maize hybrid (Co H (M) 8) grains

Abstract

The study focused on the effect of liquid and powder formulations of biofertilizer consortia developed for NPK and NPKZn nutrition using *Azospirillum*, phosphobacteria, potash bacteria and zinc solubilizing bacteria on the proximate nutrient component analysis of maize grains and to evaluate their potential as nutrient-rich sources for both human and animal consumption. Cereals, particularly maize, is one of the globally utilized and significant crops, contributing substantially to human diet, as animal feed, substrate for fuel and occupying a considerable portion of agricultural land. The nutritional composition of maize, encompassing starch, carbohydrate, protein, crude fiber and ash plays an important role in dietary planning and epidemiological research. The experiment involves different treatments, each representing a specific biofertilizer consortium application method, such as liquid and powder formulations. The liquid formulation of NPK and NPKZn biofertilizer consortia applied through seed treatment and powder formulation of NPK and NPKZn biofertilizer consortia applied through seed coating and compared with the existing recommendation in maize crop. The results indicate variations in moisture content, protein levels, starch yield, carbohydrate composition, ash content, and crude fiber among the treatments. Notably, seed coating with NPKZn biofertilizer consortia in powder form, stands out with promising attributes, including high protein content (8.08%), significant starch yield (69.88%), and balanced amylose to amylopectin ratio. The findings contribute valuable insights for selecting optimal biofertilizer consortia formulations to enhance the nutritional quality of maize, crucial for both agricultural and dietary considerations.

Keywords: Maize, NPK and NPKZn biofertilizer consortia, Proximate nutritional composition

1. INTRODUCTION

Cereals stand out as the most commonly cultivated and consumed crops globally, as they play a crucial role in supplying a significant portion of the calories in human diets and account for over half of the world's agricultural land area (approximately 45% and 54%, respectively) on a global scale [1, 2]. Among cereals, maize (*Zea mays* L.), recognized as a highly adaptable and continually evolving cereal crop, thrives in diverse agroclimatic conditions and holds a significant position within the Poaceae family, serving as a dietary staple in numerous countries worldwide [3]. Indigenous farmers are credited with a remarkable achievement in plant breeding the domestication and diversification of maize. Archaeological evidence traces the initial cultivation of maize to Mexico and Central America [4]. Renowned as the "queen of cereals" globally, maize stands out due to its unparalleled genetic production potential. Besides being a crucial staple, maize finds extensive industrial use, particularly in the production of high fructose corn syrup [3]. Maize grain, constituting a significant portion of broiler feed (60%), is utilized for non-ruminants in its whole form [5]. Notably, its high starch and low fibre content contribute to a superior conversion ratio to meat and eggs compared to other grains. In tropical regions, there's a preference for yellow maize in animal feed. Maize holds pivotal importance as a raw material in the starch, feed, and food industries, providing enhanced health benefits when incorporated into food processing and feeding [6].

Additionally, maize nutritional composition plays a vital role in nutritional planning and supports epidemiological research [7]. Maintaining a safe moisture level of 12% to 14% when wet is recommended for cereal grains, including maize [8]. Maize seeds encompass moisture (11.6-20.0%), ash (1.10-2.95%), protein (4.50-9.87%), fat (2.17-4.43%), fibre (2.10-26.70%), and carbohydrates (44.60-69.60%) [1, 6]. The versatility of maize is evident in its various products, such as whole corn, corn flour, corn starch, corn gluten, corn syrup, tortillas, tortilla chips, polenta cornmeal, corn oil, popcorn, and cornflakes. Maize processing is carried out by three primary industries: wet millers for starch, sweeteners, and maize oil; dry millers for maize flour and grits; and distillers for beverage and bioethanol production. The rising demand for maize as a carbohydrate source in the processing sector has led to the expansion of breeding programs focusing on hybrids with specialized features for distinct uses [9]. Regular consumption of maize has been associated with facilitating the removal of harmful dietary components and expediting food transit through the gut. It also plays a protective role in the digestive system, reducing stomach acidity, and enhancing gallbladder function [10, 11]. Despite limited research on comparing the nutritional content of different biofertilizer methods used on maize seeds in farmers' fields, this study aims to explore the proximate composition of various biofertilizer consortium techniques applied to maize seedlings and their quality. Understanding these variations will aid in selecting optimal biofertilizer consortiums (NPK and NPKZn) in liquid or powder formulations for both human and animal consumption.

2. MATERIALS AND METHODS

2.1. Plant material

Maize seeds COH(M)8 cultivar, sourced from the Department of Millets at the Center for Plant Breeding and Genetics, Tamil Nadu Agricultural University in Coimbatore, were utilized for the study.

2.2. Development of biofertilizer consortia for maize

Two distinct biofertilizer consortia (NPK, NPKZn) were developed in two different formulations (liquid and powder) with the aim of enhancing the efficiency of applied fertilizers and augmenting the availability of applied essential nutrients (nitrogen, phosphorus, potassium, and zinc) to the soil in the form of urea, single super phosphate, muriate of potash and zinc sulphate for maize cultivation. The liquid formulations of biofertilizer consortia were developed using the standard biofertilizers viz., *Azospirillumbrasilense* (Sp7), *Bacillus megaterium* (Pb1), *Paenibacillus mucilaginosus* (KRB9), and *Pseudomonas chlororaphis* (ZSB15) normally recommended for maize cultivation with respect to NPK and NPKZn nutrition. Using these liquid formulations of biofertilizer consortia, powder formulation was developed with carrier. The liquid formulation of NPK &NPKZn consortia was used for seed treatment of maize; whereas the powder formulation is used for seed coating of maize by seed coating mixture [12]. These biofertilizer consortia (NPK &NPKZn) coated maize seed is a kind of ready to use input. A field trial was taken in maize with five different treatments. The liquid biofertilizer consortia formulation was used for seed treatment of maize; whereas powder formulation of consortia used for seed coating of maize. These two consortia were compared with the existing recommended practice and farmer's practice. This study is mainly to investigate and compare the effect of two different formulations of NPK and NPKZn consortia on enhancing the nutritional parameters of maize grain. Because, in general biofertilizers enhance the quality of the farm produce.

2.3. Sample preparation for proximate analysis

After harvesting the maize cobs, the grains were removed and used for the entire analysis. Initially, the maize grains were dried up to 12% moisture content and the dried maize grain samples further powdered and passed through a 60-mesh sieve. The powdered samples were packed in a high-density polyethylene (HDPE) cover stored at room temperature and used for the entire study.

2.3.1. Estimation of Moisture content

Moisture Content was determined by the method described previously[13, 14]. Moisture is a critical component of food. The moisture content of any food is determined not only to assess the chemical makeup of the food item on a moisture-free basis, but also to determine the shelf life of the product. A dry and weighted petri plate held a 10 g sample. At regular intervals, the petri dish containing the material was weighed until a steady weight was attained. The percentage of moisture was calculated using the following formula:

$$\text{Moisture}(\%) = \frac{W_1 - W_2}{W} \times 100$$

where, W_1 = weight of sample with crucible (g),

W_2 = weight of dried sample with crucible (g), and

W = weight of wet sample (g).

2.3.2. Estimation of Carbohydrate

The carbohydrate was estimated by anthrone method [15]. In a boiling tube, 100 mg of maize grain sample was taken and added with 5 ml of 2.5 N HCl. This mixture was then hydrolyzed in a boiling water bath with for 3 hours and cooled to room temperature. In order to remove the effervescence, the hydrolyzed mixture was then neutralized with solid sodium carbonate and made up the volume to 100ml for centrifugation. After centrifugation, from the supernatant 0.5 and 1mL of sample aliquots were taken for assessment and compared with the standards taken at 0 (Blank), 0.2, 0.4, 0.6, 0.8, and 1ml. The sample reagents taken at different volume were made up to 1 ml with fresh water and added with 4mL of anthrone reagent. This reaction mixture was then kept in a boiling water bath for eight minutes and cooled quickly. The resultant colour developed after reaction from green to dark green was read at 630nm and compared the absorbance against the standard concentration (glucose) taken on X axis. From the standard graph, the quantity of carbohydrate was arrived using the following formula.

$$\text{carbohydrate (g/100g)} = \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

2.3.3. Estimation of starch

To estimate starch, first the sugars in the maize grain sample has to be eliminated [15]. To eliminate the sugars, 0.5 g of maize grain sample was taken, homogenized in 80% ethanol and the residue was obtained by centrifugation. The residue was rinsed with 80% ethanol until it becomes colourless with addition of anthrone reagent. Once the residue becomes colorless, then it was carefully dried in a water bath. To the dried residual substance, 5.0 mL of distilled water and 6.5 mL of 52% perchloric acid was added and performed the extraction at 0°C for 20 minutes. Now the sample contents were centrifuged, and repeated the extraction with fresh perchloric acid. After extraction, all the supernatants were pooled to yield a total volume of 100 ml. From the supernatant 0.2 ml was taken and diluted to 1 ml with water. Simultaneously, standards were also taken at 0.2, 0.4, 0.6, 0.8, and 1 mL of water in each tube and added with 4 mL of anthrone reagent for reaction and kept in a boiling water bath for eight minutes. After the reaction, the sample was cooled rapidly and measured the intensity of color changed from green to dark green at 630 nm and the amount of glucose was calculated as per the following equation,

$$\text{Starch (\%)} = \text{glucose (\%)} \times 0.9$$

2.3.4. Estimation of Amylose

The powdered sample of 100 mg was taken and added with 1 ml of distilled ethanol added, followed by 10 ml of 1 N NaOH, and allowed it to stand overnight. The extract volume was then raised to 100 ml. From that, 2.5 ml of the extract was taken and to that 20 ml of distilled water, and few drops of

phenolphthalein were added. To this reaction mixture, 0.1 N HCl was added drop by drop until the pink color completely disappears and the volume was adjusted to 50 ml with 1 ml of iodine reagent, and read at 590 nm. Standard amylose solutions of 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, and 1 ml were also taken, and allowed till it develop the color as in the example. Using a standard graph, the quantity of amylose in the sample was calculated. For a blank, 1ml of iodine reagent diluted in 50ml of distilled water was used.

Absorbance corresponds to 2.5ml of test solution = 'x' mg amylose

100 ml contains = ('x' ÷ 2.5) × 100mg amylose = % amylose

Subtracting the amylose content from the starch content provides a quantity of amylopectin.

2.3.5. Estimation of Protein content

The AOAC standard technique was used to determine the crude protein content[16]. Protein content was calculated using three stages: digestion, distillation, and titration.

i) N₂ content calculation:

% of N₂ = Burette reading × Normality of H₂SO₄ × mL equivalent of N₂

In this case, the Normality of H₂SO₄ is 0.2 and the ml equivalent of N₂ is 1.4.

ii) Protein content calculation:

% Protein = % of N₂ × Protein factor

In this case, Protein factor = 6.25.

2.3.6. Estimation of Crude fibre

The crude fibre content was determined using the wende approach through the acid-alkali digestion technique[17]. Fibre, consisting of insoluble vegetable matter such as cellulose, hemi-cellulose and lignin, is resistant to digestion by proteolytic or diastatic enzymes and can only be utilized through microbial fermentation. In this method, three grams of a moisture and fat-free maize grain samples from different treatments were placed in a 500 ml beaker, boiled for 30 minutes with 200 ml of 1.25 percent sulphuric acid sodium hydroxide solution. The samples were boiled, then filtered through muslin cloth. The residue was then rinsed with hot distilled water until the alkali was no more visible, and then 50 ml of alcohol and ether were used to wash it. The residue was then placed in the crucible (W₁) and dried in an oven at 130°C for 2 - 3 hours before being cooled and weighed (W₂). In a muffle furnace at 600 °C for 2-3 hours, a crucible containing dry residue was cooled, and weighed again (W₃). Crude fibre was calculated using the method below;

$$\text{Crude fibre(\%)} = \frac{(W_2 - W_1) - (W_3 - W_1)}{\text{Weight of sample}} \times 100$$

Where, W₁= Weight of empty crucible

W₂= Weight of crucible with dry residue

W_3 = Weight of crucible with heated residue

2.3.7. Estimation of Ash content

The ash content of a maize sample was determined using the AOAC standard procedure [16]. Five gram of powdered maize sample was taken in a pre-weighed crucible and placed in a muffle furnace at 550°C to 600°C for 5-6 hours. After complete ashing, the crucible was cooled and dried in a desiccator before being weighed. From the weights recorded the present ash content was calculated by the following equation.

$$\text{Ash (\%)} = \frac{W_1 - W_2}{W} \times 100$$

where, W_1 = weight of ash with crucible (g)

W_2 = weight of empty crucible (g)

W = weight of sample (g).

2.4. Statistical analysis

A statistical analysis was conducted to determine the nutritional composition of maize in a Completely Randomized Design (CRD) with five replications and analyzed using statistical software (SPSS windows version 20). ANOVA was performed on triplicate values. Mean comparisons were performed using Duncan's multiple range tests for significant effect at least of $p < 0.05$.

3. RESULTS AND DISCUSSION

The proximate analysis of maize grains evaluated under five different treatments (T_1 to T_5), revealed variations in key nutritional parameters.

3.1. Moisture content

The moisture content in food holds significant economic importance for both producers and consumers, as it exhibits an inverse relationship with the dry matter present. This factor is also pivotal for ensuring the stability and overall quality of food products. The results from the present study revealed that the moisture content recorded ranges from 3.6 % to 4.0 % (Table 1; Fig 1). The significantly maximum moisture content of 4.0 % was observed in maize seed treated with NPK liquid biofertilizer consortia (T_2). In contrast to Oluwalana's findings, who recorded a moisture content ranging from 9.85% to 11.35% in maize, the current study observed a decreased level of moisture content irrespective of all the treatments (3.6 to 4.0 %)[18]. This itself showed a positive sign about the quality of maize grain to be used as food or feed. Because, higher moisture levels tend to decrease the shelf life of the maize; and the observed lower moisture content -a favourable characteristic for prolonged storage. Moreover, grains with elevated moisture levels are susceptible to quick deterioration due to issues like mold growth and insect damage, underscoring the need for careful consideration of moisture content in food processing and consumption[19, 20].

3.2. Carbohydrate

Maize is recognized for its high carbohydrate content, providing energy, facilitating the utilization of body fats through metabolic processes, and supporting the proper functioning of the intestinal tract. The analysed maize grain samples showed carbohydrate content ranged from 67.76 to 74.40 %. This is in accordance with the results previously reported[21, 22]. The study also revealed a significant difference in carbohydrate content among the five treatments (Table 1; Fig 2), with carbohydrate levels ranging from 69.25% to 71.13%. The maximum carbohydrate content of 71.13% was noted in the maize seed coated with NPKZn (T₅) biofertilizer consortia, compared to the carbohydrates content in maize seed coated with NPK biofertilizer consortia (T₄), maize seed treated with NPKZn liquid biofertilizer consortia (T₃), and uninoculated (T₁) recorded 70.89%, 70.89% and 69.28%, respectively. In accordance with the present study findings, Ullah and his co-workers also recorded a carbohydrate percentage ranging from 69.659% to 74.549% in maize[6].

3.3. Estimation of starch

Starch is the main carbohydrate reserve in plants and an important part of our nutrition. The mean starch yield of the maize treatments ranged from 67.24% to 69.88% (Table 1; Fig 2). The starch obtained from maize seed coated with NPKZn biofertilizer consortia (T₅) was significantly ($P < 0.05$) higher (69.88 %) than maize seed coated with NPK biofertilizer consortia (T₄) recorded 68.36 which is on par with the other two liquid formulation of biofertilizer consortia treatments (T₃ & T₂). The starch yield is less than the values obtained for potato and corn starch, which were 93.4% and 96.5% respectively[23]. While the wide range of starch content among the cultivars is interesting, further information regarding specific starch components should be more helpful in formulating an effective strategy for their further utilization. The amylose percentage ranges from 20.22% to 21.98%, while amylopectin percentage varies from 46.47% to 47.90%, indicating different starch structures.

3.4. Estimation of protein content

Protein is the most important biochemical character; since it contributes to the synthesis of nitrogen-containing compounds, including antibodies and enzymes, which are essential for normal physiological functions. In the present study, compared to uninoculated control all the biofertilizer consortia formulation treatments recorded an increase in crude protein content (7.70 to 8.08%) (Table 1; Fig 1). The significantly maximum value of protein content 8.08% was observed in maize seeds coated with NPKZn biofertilizer consortia (T₅) maize grains, followed by seed coating with NPK biofertilizer consortia (T₄) recorded high protein. Uninoculated maize seeds (T₁) registered a minimum protein content of 7.51%. Similar to this study, the effect of *Azospirillum* and *Azotobacter* was found to increase the protein content in maize as 6.34 & 7.00 % respectively[24]. Various studies have documented protein levels in maize hybrids ranging from 7.77% to 13.84%[25].

3.5. Estimation of crude fibre

Crude fibre, primarily composed of cellulose and hemicellulose, offers favourable effects in humans by enhancing water retention capacity as food moves through the digestive tract. A diet abundant in crude fibre is considered healthful [26]. as it contributes to the production of larger and softer faeces. The results of crude fibre analysis in various treatments ranged from 1.82% to 1.94% (Table 1; Fig 1).

The maximum crude fibre content, 1.94%, was observed in maize seeds coated with NPKZn biofertilizer consortia (T₅), followed by maize seeds coated with NPK biofertilizer consortia (T₄) at 1.92%, while the minimum crude fibre content, 1.82%, was noted in uninoculated maize seeds (T₁). The crude fibre percent within the range of 0.80% to 2.32% was reported closely aligning with the findings of the present study[6].

3.6. Estimation of ash content

The analysis of foodstuffs for ash content percentage serves to identify the non-organic matter component within the dry matter, representing the residue after the organic matter undergoes processes like oven drying, ignition, or complete oxidation. This ash content provides a rough estimate of the overall mineral content present in the food. In the case of maize seeds under different treatments, the percentage ash content ranged from 1.98% to 2.15% (Table 1). Comparable findings of 0.70% to 2.50% in various maize hybrids were previously reported[27-30]. Maziya-Dixon and co-workers also reported results within the range of 1.4% to 3.3%, demonstrating close consistency with the ash content values observed in the present investigation[31].

4. CONCLUSION

This research investigated the impact of liquid and powder formulation of NPK and NPKZn biofertilizer consortia, on the proximate nutritional composition of maize grain under different treatments. In general, bio-organic fertilization improves soluble sugars, starch, carbohydrates, protein, and amino acid contents in maize seeds. In the present study also revealed notable variations in moisture content, carbohydrate composition, starch content, amylose and amylopectin ratios, crude protein, crude fibre, and ash content among the treatments. Among the treatments, maize seed coating with powder formulation of NPKZn biofertilizer consortium, emerges as particularly promising, exhibiting favourable attributes such as low moisture content, high carbohydrate and starch content, balanced amylose to amylopectin ratio, elevated crude protein, and moderate crude fibre and ash content. These findings contribute valuable insights into the potential of biofertilizer consortia to enhance the nutritional quality of maize, with implications for both agricultural practices and human dietary considerations. The study provides a comprehensive understanding of the proximate nutritional changes induced by different biofertilizer treatments, offering practical guidance for optimizing maize cultivation strategies.

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Table 1: Effect of biofertilizer consortia (NPK and NPKZn) on the proximate nutritional composition of maize grain

S. No.	Treatments	Moisture (%)	Carbohydrate content (%)	Starch content (%)	Amylose (%)	Amylopectin (%)	Crude protein (%)	Crude fibre (%)	Ash content (%)
1	T ₁	3.70 (±0.05) ^b	69.28 (±0.31) ^a	67.76 (±0.58) ^b	20.22(±0.04) ^b	47.54 (±0.08) ^a	7.51 (±0.04) ^b	1.82 (±0.03) ^b	1.98 (±0.03) ^c
2	T ₂	4.00 (±0.05) ^a	69.25 (±0.92) ^a	67.24 (±0.09) ^b	20.76 ±0.23) ^b	46.47(±0.73) ^a	7.70 (±0.03) ^b	1.87(±0.01) ^{ab}	2.03 (±0.03) ^{bc}
3	T ₃	3.80 (±0.06) ^b	70.89 (±0.29) ^a	68.22 (±0.12) ^b	20.45(±0.05) ^b	47.77 (±0.75) ^a	8.02 (±0.02) ^a	1.90 (±0.00) ^a	2.11 (±0.02) ^{ab}
4	T ₄	3.72 (±0.01) ^b	70.89 (±0.36) ^a	68.36 (±0.30) ^b	21.84 ±0.27) ^a	46.53 (±0.06) ^a	8.03 (±0.11) ^a	1.92 (±0.01) ^a	2.07(±0.01) ^{abc}
5	T ₅	3.60 (±0.05) ^b	71.13(±0.02) ^a	69.88 (±0.40) ^a	21.98 ±0.12) ^a	47.90 (±0.13) ^a	8.08 (±0.07) ^a	1.94 (±0.02) ^a	2.15 (±0.03) ^a
	Mean	3.76	70.29	68.29	21.05	47.24	7.87	1.89	2.07

Mean with same alphabet in the same column are not significantly different from each other at p<0.05

T₁ –Uninoculated maize seeds (control), T₂–Seed treatment with liquid formulation of NPK biofertilizer consortium, T₃–Seed treatment with liquid formulation of NPKZn biofertilizer consortium, T₄–Seed coating with powder formulation of NPK biofertilizer consortium, T₅– Seed coating with powder formulation of NPKZn biofertilizer consortium.

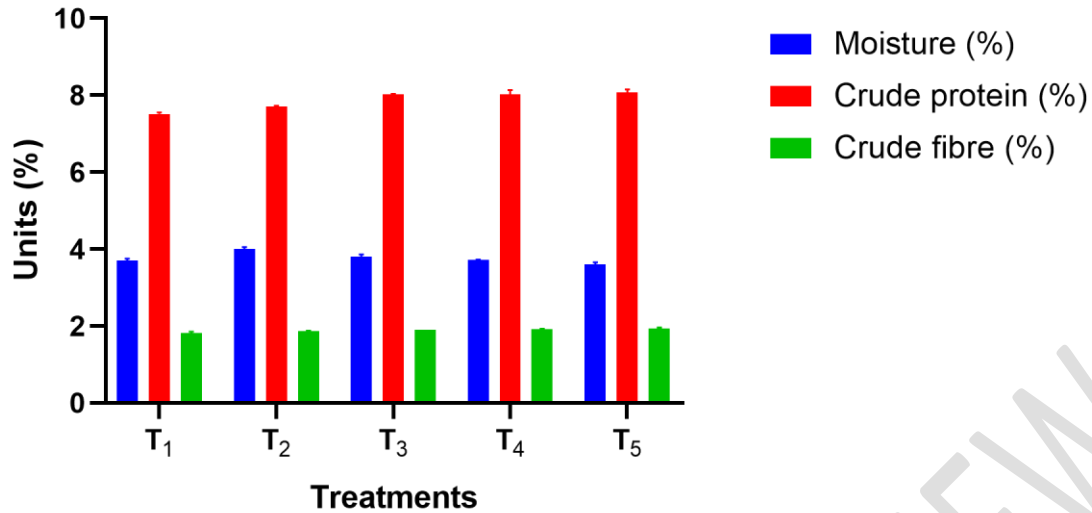


Fig. 1.Effect of biofertilizer consortia (NPK and NPKZn) on the moisture, crude protein and crude fibre content of maize grain. T₁ –Uninoculated maize seeds (control), T₂–Seed treatment with liquid formulation of NPK biofertilizer consortium, T₃–Seed treatment with liquid formulation of NPKZn biofertilizer consortium, T₄–Seed coating with powder formulation of NPK biofertilizer consortium, T₅– Seed coating with powder formulation of NPKZn biofertilizer consortium.

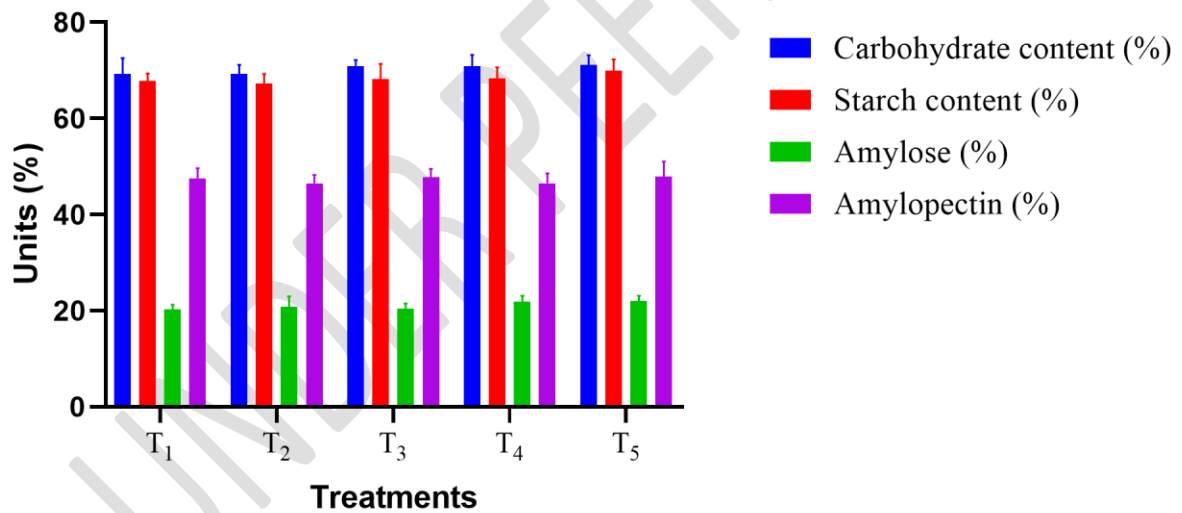


Fig. 2.Effect of biofertilizer consortia (NPK and NPKZn) on the carbohydrate, starch, amylose and amylopectin content of maize grain. T₁ –Uninoculated maize seeds (control), T₂–Seed treatment with liquid formulation of NPK biofertilizer consortium, T₃–Seed treatment with liquid formulation of NPKZn biofertilizer consortium, T₄–Seed coating with powder formulation of NPK biofertilizer consortium, T₅– Seed coating with powder formulation of NPKZn biofertilizer consortium.