



Exploring the utilities of Rice Straw black liquor (Part XIV): Assessment of a previously prepared biopesticidal formulation for enhancing Faba bean Plant Growth

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Abstract

The current study investigates to what extent a bioactive formulation, previously shown to suppress soil-borne plant pathogens, can affect the growth of the Faba bean plant. This formulation is expected to inhibit harmful pathogens and promote plant growth by gradually releasing essential nutrients derived from the co-digestion of two bio-wastes, namely the pulping liquor of rice straw and chicken feathers.

The experiment tested different ratios of formulation to soil: a control group with no formulation and three treatment groups with increasing formulation concentration (0.125%, 0.25%, and 0.5%). Each treatment was replicated three times. As expected, the concentration of nitrogen (N), phosphorus (P), and potassium (K) in the soil increased with higher formulation percentages. The highest nutrient content was observed in the 0.5% formulation treatment.

Furthermore, applying this formulation resulted in the highest uptake of essential nutrients (nitrogen, phosphorus, and potassium, or NPK) in both the shoots and grains of the plants. Additionally, there was a significant increase in the protein content of the grains across all varieties during the studied season.

This suggests a potential benefit of such dual-functional compost (DFC) in the soil, releasing a combination of components from the digested feathers and pulping liquor. These breakdown products formed a valuable and economical biofertilizer while also protecting plants as a biopesticide.

Keywords: Black liquor; Chicken Feathers; Biofertilizer; Biopesticide; Rice Straw.

1. Introduction

Faba beans are a widely cultivated versatile crop, grown both for their nutritious seeds and to improve soil health by being used as a cover crop. In Egypt, it has become a famous dish for human nutrition daily due to its richness in seed protein contents and carbohydrates [1]. Feeding a growing population requires maintaining high crop productivity. The growth and productivity of the Faba bean are affected by many factors, including chemical and organic fertilizers, where nitrogen, phosphorus, and potassium (NPK) fertilizers are necessary to increase production. Faba beans rely heavily on phosphorus for healthy growth and nitrogen fixation. This essential nutrient fuels the plant's energy transfer, promotes rapid root development, and forms the building blocks of cells (membranes and nucleic acids). Phosphorus directly impacts seed yield and quality and is crucial for flowering and fruit formation [2]. In addition, potassium (K) is a vital nutrient for plant metabolism, activating many enzymes and playing a key role in water management [3]. Faba bean, in particular, benefits greatly from K application, especially during dry seasons [4]. Studies have shown that sufficient K availability enhances the Faba bean's ability to fix nitrogen from the air [5, 6]. This translates to healthier plants and increased grain yield.

While synthetic fertilizers have boosted yields globally, their overuse can pollute water systems through a process called eutrophication. Therefore, organic materials like compost and humic substances as sustainable alternatives are looked at to ensure food security without harming the environment. These organic options hold promise as natural growth stimulants for crops [7].

One promising approach involves utilizing feathers, a waste product from the poultry industry, with millions of tons produced worldwide annually. These feathers often pose a challenge for waste disposal. Incineration and landfilling, the common disposal methods, can harm the environment. Preparation of biofertilizers using chicken feather wastes is attracting the focus of many research scientists. Feather meal is a cheap and easily available source of nitrogen (15% N) and may serve as a potential biofertilizer [8]. The plant growth-promoting activity of protein hydrolysates could also be effectively applied in agriculture [9]. Thus, microbial degradation of feather represents an alternative for the development of slow-release nitrogen fertilizers. Feather digestion emerges as a potential solution for creating slow-release nitrogen fertilizers, a more eco-friendly alternative. By feathers digestion, amino acids and peptides are

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produced [10], which serve as building blocks for beneficial plant growth metabolites like the natural plant growth hormone named indole acetic acid (IAA). Gomaa *et al.* [11] reported that the heaviest nodule weight was recorded due to the bioorganic treatment of chicken compost, which reached 3.73 g/plant against 1.81 g/plant for the positive control. The percentage of increases for dry weight of plants (g/plant) ranged from 2 to 41% over the positive control owing to the different tested bioorganic treatments.

In the same context, rice straw is one of the most important agricultural wastes that are intensively produced all over the world. Although rice straw is rich in bioactive components, only a small amount is used for industrial applications, while the rest is disposed of improperly *via* open burning, which causes environmental and health problems.

In an attempt to benefit from rice straw and the important components it contains, we have published many studies [12–20] aiming to end with zero waste and find sustainable bioactive alternatives to the imported and petroleum-derived ones. Recently, we have reported the preparation of a new biopesticide formulation [18], able to control root rot and root knot infection in green beans *via* digesting chicken feathers with the alkaline black liquor of rice straw. In the current study, we aim to elucidate the effect of this low-cost, eco-friendly formulation on the yield productivity and nutritional contents of Faba bean, hoping not to harm the plant or inhibit its growth. If it further enhances Faba bean growth, we will get green dual-functional compost as pesticides/biofertilizer. Feathers and black liquor of rice straw are rich in essential nutrients (nitrogen, phosphorus, potassium, and sulfur) and have the potential to significantly enhance soil health and fertility. These materials, when co-digested to produce an organic fertilizer, are expected to promote robust plant growth and increased yield. The underlying mechanism involves the release of organic compounds like sugars, amino acids, humic acid, and organic acids into the soil. These compounds directly nourish plants, stimulate enzymatic and hormonal processes, and indirectly improve nutrient availability by optimizing soil pH. Furthermore, black liquor's antioxidant properties, attributed to compounds such as phenolics, lignin, and ferulic and coumaric acids, safeguard plants from oxidative damage. Such a mild process to digest feathers utilizing the alkaline, non-toxic liquor affords compost that has nitrogenous and other plant growth-promoting nutrients.

2. Materials and Methods

2.1. Design of the Chemical processes:

A mild alkaline solar pulping process produces nontoxic black liquor (pH = 10). Firstly, 75 g of dry chicken feather were allowed to be digested by 1.5 L of black liquor (pH = 10) and left to be concentrated to 750 mL, where viscous hydrolysate was obtained. After neutralization by 50% H₂SO₄, 28 g of Ca-lignate/silicate were added to the hydrolysate with continuous stirring. The pH was then maintained at 8 using H₂SO₄. After adding ethanol (97%), the pH was then maintained at 5 using H₂SO₄, and the mixture was left overnight. On the next day, the mixture was filtered using a fabric sac. The precipitated solid was then mixed well with 10 g of ground rice straw to obtain a

homogenous solid. The chemical composition of the prepared formulation is reported in Table 2.

2.2. Plant cultivation:

Faba bean plants were grown in a greenhouse at the National Research Centre in Dokki, Giza, Egypt. The experiment assessed the influence of a reported biopesticidal formulation [18] as green compost on plant growth, nutrient content, and yield productivity. The experiment consisted of four groups: a control group and three treatment groups (T1, T2, and T3) using concentrations of 0.125%, 0.25%, and 0.5%, respectively. Each treatment was repeated three times. Twelve cylindrical pots, measuring 35 cm in diameter and 50 cm in depth, were used. Each pot contained 30 kilograms of dried silty clay loam soil, whose main physico-chemical characteristics are reported in Tables 1 and 2, employing the methodologies outlined by Cottoniee *et al.* [21]. Two plants per treatment were harvested, washed, and dried in an electric oven at 70 °C. The dried plants were then ground into a fine powder using a stainless steel mill. The seeds were ground and subsequently analyzed for nutrient content using the methods described by Cottoniee *et al.* [21].

Sowing was conducted on November 11, 2023, at a planting density of 10 plants per plot, arranged in two rows. Each treatment was replicated three times at each sampling point. Data was collected 45, 90, and 120 days after planting.

Harvested samples were transported to the laboratory, cleaned with tap water to remove dirt and clay, and then separated into leaves, roots, and seeds. Seed harvesting and analysis were performed on three plants per pot at the fresh pod stage (March 14–April 9) and at the full ripening stage (May 21).

Morpho-physiological and quality parameters were determined. Seeds were milled and analyzed for antioxidant activity, total phenolic compounds, and protein content.

2.3. Faba bean milling:

Dry Faba bean seeds were milled to whole flour by means of a laboratory mill equipped with a 1-mm sieve (Cyclotec Sample Mill, Tecator Foss, Hillerød, Denmark).

2.4. Plant analysis

Bean plant samples were dried in an oven at 70° C till constant weight and the dry weight was recorded. The N, P, and K concentrations were determined in these dried plant samples at two stages: booting in the whole plant and at harvest in both separated organs of straw and seeds.

The dried plant samples were ground into a powder and 0.2 grams of each sample was weighed and wet digested. N, P and K nutrients were measured in the digestive extract and their percentages were calculated on oven dry weight. Nutrients determination was performed as follows:

- Total N, P and K (%) were determined according to the methods described by Mertens (2005a & b) [22].
- Total nitrogen was determined by the Kjeldhal method, while total protein was calculated by multiplying total nitrogen x 6.25 [23].
- Total calcium was determined using Gallenkamp flame photometer as described by Cottoniee *et al.* [21].
- Total carbohydrates % was determined according to Ranganna [24].

3. Results and Discussion

Results revealed that the addition of the prepared formulation resulted in significant positive influence on the growth of Faba bean plant as expected.

3.1. Effect of the applied treatments on fresh and dry weight of Faba bean plants:

Fresh weight:

As depicted in Figure 1, T1 treatment showed a slight increase in fresh weight for both leaves (52.54 g vs. 52.00 g) and roots (22.49 g vs. 22.39 g) compared to the control. A significant enhancement in leaf (60.4 g) and root (24.02 g) fresh weight was observed with T2 treatment compared to the control. Similarly, T3 treatment followed the same trend; leading to increased fresh weight for both leaves (61.52 g) and roots (25.50 g).

Table (1) Analytical properties of investigated soil:

1- Mechanical analysis : 0-30 cm													
Sand %		Silt 20-2 μ %	Clay%<2 μ	Soil texture									
Course >200 μ	Fine>200-20 μ												
7.8	5.6	50.8	35.8	Silty clay loam									
2- Chemical analysis:													
No. Soil	pH	EC dSm ⁻¹	CaCO ₃ %	O.M %	Soluble cations (meq/L)				Soluble anions (meq/L)				
	1:2.5				Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	CO ₃ ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻	
	8.17	0.99	0.26	0.63	4.26	0.64	2.00	3.00	-	0.15	2.50	7.25	
Macronutrients													
No. Sample	Total%				Available(mg/100gsoil)								
	N	P	K	Ca	N	P	K	Ca					
Soil 0-30	0.16	0.37	0.54	0.50	1.25	2.75	3.84	0.96					

Table (2) chemical analysis of DFC compost:

Chemical analysis:													
No.	pH 1:2.5	EC dSm ⁻¹	CaCO ₃ %	O.M %	Soluble cations (meq/L)				Soluble anions (meq/L)				
					Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	CO ₃ ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻	
DFC compost	8.51	19.05	1.30	0.80	103.7	5.84	14.75	66.3	-	21.5	44.25	124.75	
Total %													
Total %				Available (ppm)									
N	P	K	Ca	N	P	K	Ca						
2.91	0.32	0.50	1.76	159.9	111.3	1261.3	229.6						
% on air dried basis (moisture)	hydrolysable protein (ammonia & Nitrogen source)	Crude fibers (hydrolysable lignin)	Crude fat	Ash (silica & inorganics salts)	Total carbohydrate (hemicellulose/cellulose as carbon sources)								
5.88 %	28.80 %	6.84 %	1.51 %	32.22 %	24.75								

Moreover, the application of these different treatments significantly impacted the fresh weight of mixed pods and seeds. As presented, T1 increased this weight to 19.37 g, while T2 boosted it to 47.15 g. The maximum weight of 54.79 g was achieved with T3. These findings align with Ahmed and El-Abagy [25], who linked variations in growth characteristics among Faba bean cultivars to differences in nodule formation, suggesting a primary reliance on nitrogen fixation for growth. Additionally, these differences were attributed to variations in partition and migration of photosynthases between cultivars and the endogenous.

Dry weight:

The treatment application also influenced the dry weight of plant components. As indicated in Figure 2, T1 resulted in a slight increase in leaf (11.73 g vs. 11.66 g) and root (2.83 g vs. 2.69 g) dry weight compared to the control. T2 treatment further enhanced leaf (12.82 g) and root (2.86 g) dry weight. The most substantial increase in both leaf (17.15 g) and root (3.73 g) dry weight was observed with T3 treatment.

The application of the selected treatments significantly boosted the dry weight of both pods and seeds. The T3 treatment resulted in the highest values for both, with 3.37 g for pods and 3.55 g for seeds, while the control treatments recorded 1.03 g and 1.6 g for pods and seeds, respectively. Talaat and Abdallah [26] recommended the combined use of mineral and biofertilizers with two Faba bean varieties

(Sakha-1 and Giza-40). Their research indicated that Sakha-1 exhibited significant enhancements in pod and seed count per plant, pod and seed weight per plant, seed and straw yield per feddan, and 100-seed weight.

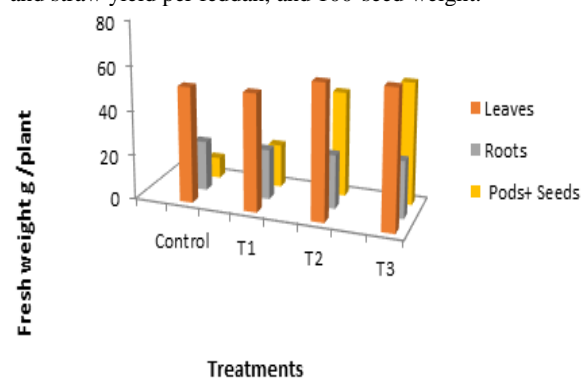


Fig. 1. Effect of the applied DFC rates on mean fresh weight of Faba bean plant.

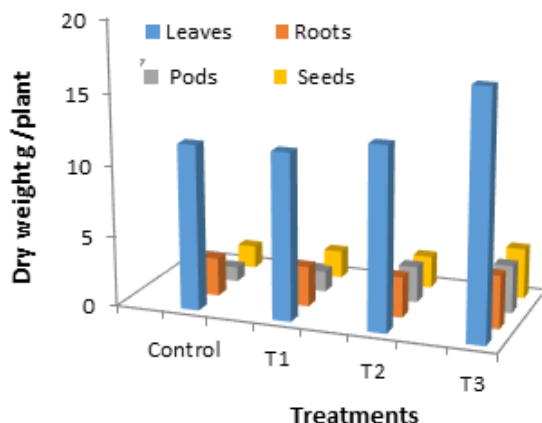


Fig. 2. Effect of the applied DFC rates on mean dry weight of Faba bean plants

3.2. Effect of the applied DFC on nutrients, carbohydrate and sugar content of Faba bean plants:

Nitrogen content:

Nitrogen is a vital primary nutrient essential for plants. As a fundamental building block of amino acids and proteins, it plays a crucial role in plant structure. Additionally, nitrogen is a component of nucleic acids, vital for cell division and reproduction. Plants rely on readily available nitrogen for growth and development [27]. Moreover, a significant decline in nitrogen levels can result in the ammonia-friction (NH_4^+) process, which converts a fraction of organic nitrogen into NH_3 and NH_4^+ ions [28].

As indicated in Figure 3, T1 treatment led to a slight increase in leaf nitrogen content (3.44% vs. 2.98%) and root nitrogen content (2.68% vs. 2.50%) compared to the control, with no impact on shoot nitrogen. Increasing the feather treatment application rate (T2) significantly elevated leaf nitrogen content to 3.78% and root nitrogen content to 3.16% compared to the control. Similarly, T3 treatment followed the same pattern, further increasing leaf nitrogen content to 4.07% and root nitrogen content to 3.29%.

The N% of both pods and seeds was significantly impacted by the applied treatments. As shown, T1 increased N content to 2.07% in pods and 2.41% in seeds compared to the control (1.80% and 1.81%, respectively). T2 further elevated these values to 2.42% and 2.73%, respectively. The highest N content was observed with T3, reaching 3.63% for pods and 3.65% for seeds.

The protein content in the studied plants was significantly enhanced by the applied treatments. T3 treatment yielded the highest protein content at 22.88%, surpassing T1 (16.56%) and T2 (18.88%). The control treatment recorded a protein content of 14.19%. The application of farmyard manure accompanied by the tested biofertilizers produced nodule numbers (96–177% over the positive control), and nodule fresh weights were greater than those recorded with the same bioorganic treatments of chicken manure (22–167% over the positive control). The obtained results could be ascribed to the high nitrogen content of chicken manure (2.02%).

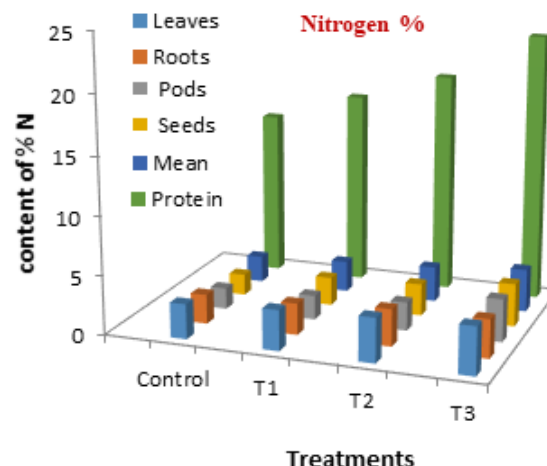


Fig. 3. Effect of the applied DFC on mean total content of nitrogen in Faba bean plants.

Phosphorus content:

Phosphorus, a crucial primary nutrient, provides essential energy for plant growth and maintenance [29]. As shown in Figure 4, applying T1 to the soils slightly elevated phosphorus (P) content in leaves (0.26% vs. 0.17%) and roots (0.16% vs. 0.13%) compared to the control. A significant increase in P content was observed in both leaves (0.59%) and roots (0.36%), with the highest application rate of amended material in T3. The mean weight of pods and seeds is significantly influenced by the application of the different treatments. As indicated in Figure 4, P content rose from 0.18% to 0.40% in pods and from 0.44% to 0.73% in seeds when comparing the control to T3 treatment. Intermediate values were found for T1 and T2. Overall, as depicted in Figure 4, T3 consistently yielded the highest phosphorous levels across all plant components.

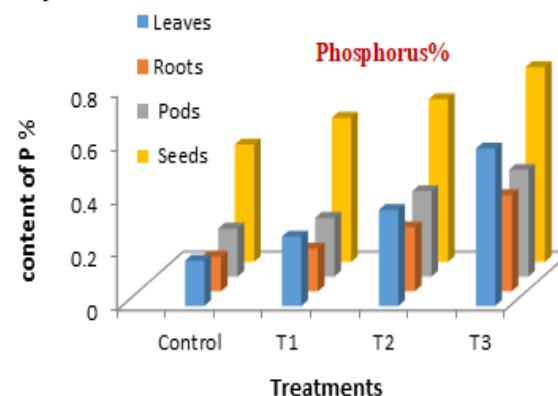


Fig. 4. Effect of the applied DFC on mean total content of phosphorus in Faba bean plants.

Potassium content:

Potassium, a crucial nutrient, plays a vital role in plant function. It activates essential enzymes and controls the opening and closing of stomata, which regulates airflow and water transpiration out of the leaves [30]. As shown from Figure 5, potassium content was significantly enhanced by all treatments compared to the control. Application of T1 led to slight increases in K content of leaves and roots (1.52% and 1.27%, respectively). A more

pronounced increase was observed with T2. However, the highest K content was achieved with T3, attaining 1.96% and 1.88% in leaves and roots, respectively. Similarly, K content in pods and seeds was significantly elevated by the treatments. While T1 increased K content to 1.87% and 1.29% for pods and seeds, respectively, and T2 to 1.99% and 1.44%, T3 demonstrated the most substantial impact, with values reaching 2.27% and 1.65%, respectively. Also, Fouda *et al.* [31] found that the content of phosphorus, potassium, and other minerals in the seeds of pea plants increased under the application of compost and farmyard manure.

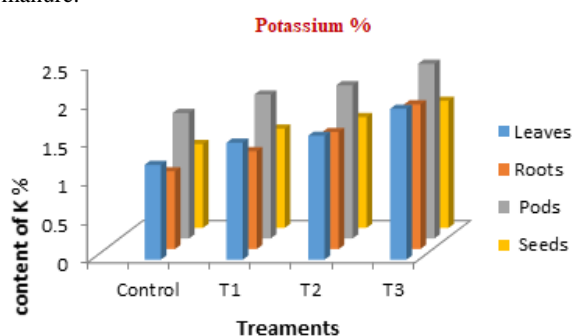


Fig. 5. Effect of the applied DFC on mean total content of potassium in Faba bean plants.

Calcium content:

Calcium content in leaves and roots was significantly enhanced by all treatments compared to the control. T1 showed a slight increase in calcium levels, while a more pronounced increase was noticed by T2. The highest calcium levels were observed with treatment T3, at 2.77% and 2.43% for leaves and roots, respectively. Regarding the mean weight of pods and seeds, results showed that Ca increased to 1.75, 1.13, and 1.93, 1.19% by application T1 and T2, respectively, compared to 1.45 and 0.93% for the control treatment, while the highest values of 2.25 and 1.57% were obtained when applied T3, respectively (Figure 6).

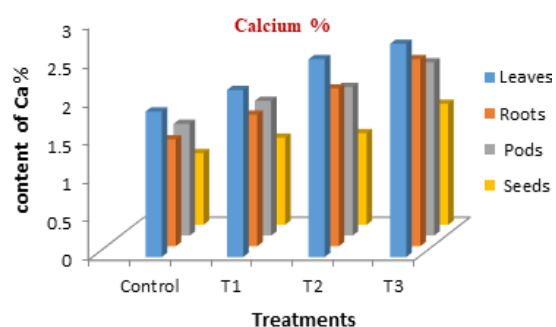


Fig. 6. Effect of the applied DFC on mean total content of calcium in Faba bean plants.

Carbohydrate content:

Results in Figure 7 showed that on mean values, application of T1 slightly increased the carbohydrate of leaves and roots to 17.15 and 1.84% compared to 15.26 and 1.50% for the control treatment. Applying T2 significantly increased the carbohydrate of leaves and roots to 19.94 and 2.11% compared to the control. It should be mentioned that the application of T3 gave the same trend of increasing the mean values of carbohydrates to 23.40 and 2.56% of both

leaves and roots, respectively. The mean values of carbohydrate in pods and seeds are significantly influenced by the application of different treatments. Results showed that the carbohydrate percentages were increased to 6.88, 23.31 %, and 7.75, 24.75 % by application T1 and T2, respectively, compared to 4.73 and 22.01 % for the control treatment, while the highest values of 9.00 and 36.75 % were obtained when applied T3. Generally, El-Mansi *et al.* [32] reported on pea that applying compost and nitrogen fertilizer increased total carbohydrate and protein in seeds, which may be due to the high growth and pod high yield of plants fertilized by nitrogen fertilizer combined with compost manure; it may be due to the availability of organic nitrogen, which ultimately resulted in better root growth and increased physical activity of roots to absorb the nutrients through decomposition of organic manure that led to an increase in their contents.

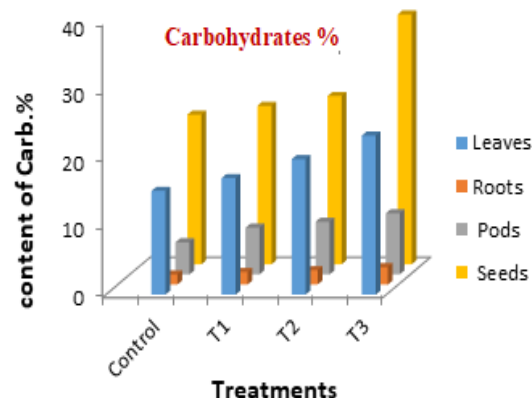


Fig. 7. Effect of the applied DFC on mean total content of carbohydrates in Faba bean plants

Sugar content:

Results in Figure 8 showed that increasing the rate of application of chicken feather in treatment resulted in increasing the sugar content of leaves and roots, with T3 exhibiting the highest trend of increasing sugar content to 1.91 and 0.64% for both leaves and roots, respectively. The mean weight of pods and seeds was significantly influenced by the application of different treatments. Results showed that the sugar contents increased with increasing the application rate, reaching the highest values of 0.58 and 5.75% when applied T3. With respect to pod total sugar content, data showed that 40 m³ chicken compost biofertilizer gave significantly greater pod total sugar content compared with control, according to Kabeel *et al.* [33].

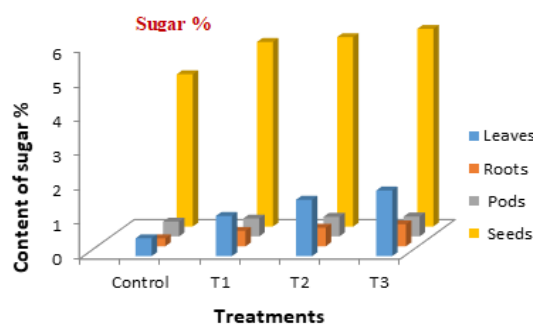


Fig. 8. Effect of the applied DFC on mean total content of sugar in Faba bean plants.

3.3. Effect of the applied DFC on the mean total content of residual nutrients in the soil after harvesting

The effect of the applied compost on the total content of residual nutrients in the soil after harvesting was studied. Results showed that the application of T1 increased the residual values compared to the control. The mean values of N, P, K, and Ca were 0.16, 0.16, 0.24, and 0.49% in control; these values increased to 0.17, 0.19, 0.36, and 0.69% when the studied soil was treated with T1. The corresponding values by application of T2 were increased again to 0.14, 0.29, 0.45, and 0.75, while the application of T3 increased the residual in the soil ecosystem to 0.18, 0.51, 0.75, and 1.40%. In other words, using such material could be a source of enhancement for the next cultivation. Results also showed that the residual contents of nutrients increased by the application of T3, followed by T2 and T1. This result may enhance preferring to apply T3 if we are looking for healthy soil for the next cultivation.

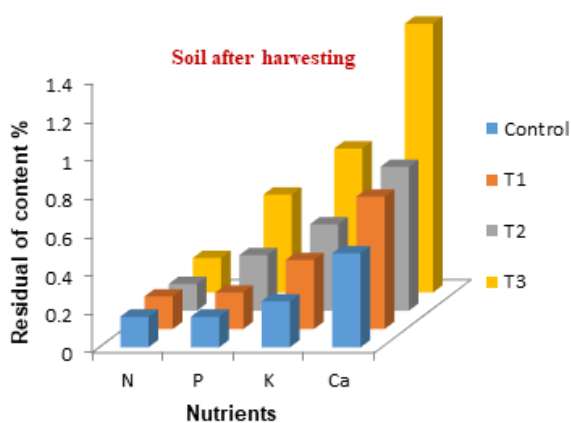


Fig. 9. Residual total nutrients in the soil after harvesting.

1. Conclusion

This study highlights the potential of utilizing the previously reported biopesticide produced from co-digested feather with rice straw black liquor as a cost-effective organic nitrogen fertilizer for plants. Enriching this formula with antioxidant and antibacterial extracts from the rice straw mild pulping process can significantly boost plant growth when combined in optimal proportions. Additionally, the phosphorus and potassium content of rice straw and nitrogen from feather waste further enhance plant nutrition and soil health. These findings underscore the value of utilizing agricultural by-products into dual-function fertilizer that not only promotes plant growth but also provides soil pest control benefits.

2. Conflicts of interest

There are no conflicts to declare.

3. Acknowledgments

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