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Nitrogen Availability Following Application of Urea and Decanter Cake/Palm Kernel Expeller Frass Produced from Black Soldier Fly Larvae

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ABSTRACT

The inefficient use of nitrogen is due to the loss of nitrogen (N) in the soil caused by a range of mechanisms, including the volatilization of ammonia, the leaching of nitrates, the runoff of soil, and autotrophic reactions. In this work, an effort was made to keep nitrogen from being lost during the urea treatment by making use of frass produced by black soldier fly larvae (BSFL) on decanter cake (DC) and palm kernel expeller (PKE). Thirty days of soil incubation was carried out to study the effects of varying concentrations of BSFL on the amount of nitrogen retained from varying concentrations of urea. In the incubation of the soil, the treatments were as follows: 300 g of soil only (T0), 300 g of soil + 4 g of urea (T1), 300 g of soil + 40 g of frass (T2), 300 g of soil + 30 g of frass+ 3 g of urea (T3), and 300 g of soil + 20 g of frass + 2 g of urea (T4). The treatments were organized using a complete randomized design (CRD), and each replication was performed three times. Incorporating BSFL frass into the soil increased pH, total carbon, organic matter, available nitrate and exchangeable ammonium, and cation exchange capacity in the soil. This is due to BSFL frass being rich in base cations. Amending urea with BSFL frass significantly increased soil cation exchange capacity (CEC) and pH because high pH contributes to a low concentration of H^+ ions in the soil and increases the possibility of NH_4^+ cations binding on the negative sites on the surface of BSFL frass. In conclusion, pH was an important regulator of net nitrification during BSFL composting.

Keywords: *Black Soldier Fly Larvae (BSFL), Urea, Frass, Organic fertilizer*

1. INTRODUCTION

Malaysia is one of the world's top producers of palm oil, with 24.49 million tonnes of palm oil and palm-based products exported in the year 2023 alone [1]. The sector's expansion leads to massive waste generation, including the palm oil mill effluent (POME), palm kernel cake (PKC), decanter cake (DC), empty fruit bunches (EFB), palm kernel shell (PKS), palm kernel expeller (PKE) and palm press fibre (PPF) [2]. Specifically, palm oil processing generated roughly 4-5 tonnes of decanter cake (DC) for every 100 tonnes of fresh palm fruit cluster processed [3]. The level of caution increases as the quantity of discarded DC builds up over time.

At the same time, there is an increasing demand for organic fertilizers. This is because of the harmful consequences that nitrogen fertilizers like urea have on the environment through their contribution to pollution. According to a study [4], improper usage of urea could trigger nitrification, releasing free hydrogen ions (H^+) in the soil and forming soil acidity. In addition, the release of hydrogen ions into the soil is another consequence of plant uptake of ammonium ions (NH_4^+). Even though synthetic fertilizers can quickly enhance crop yields, this practice can also lead to soil hardening and a decline in soil organic matter and pH, which can ultimately result in a loss of soil productivity.

The decomposition of organic waste by *Hermetia illucens* (black soldier fly, BSF) is a potential solution for managing the overabundance of palm oil processing waste like DC and PKE. At the same time, it contributes to the production of frass as an organic fertilizer source. The life cycle of BSF may be broken down into four distinct stages: the egg, larva, pupa, and adult stages [5], in which the BSF Larvae (BSFL) plays an important role in converting these organic wastes into frass. In the final step of the larval stage, the prepupae then move to a dry and adequate pupation place, where they transform into the pupa stage of the insect [6].

The decomposition of organic material is possible by BSFL due to its appetite for organic wastes, including decomposing fruits, vegetable waste, animal dung, and municipal organic wastes [7]. Additionally, its larvae are not considered pest flies and decompose organic waste from various sources. BSFL, contrary to many other pests that feed on trash, does not harbour bacteria or disease and can make *Escherichia coli* and *Salmonella* harmless [8]. BSFL has a protein content of 42.1-56.9% and a lipid content of 19-37%, which makes the amino acid and fatty acid profiles of BSFL appropriate for inclusion in the poultry diet [9].

Even after the harvesting of BSFL, a significant quantity of insect frass, often known as the excrement of the larvae, will remain. The excrement is the waste resulting from the growth of BSFL, which are often fed with dry grains of distillers with soluble, and they contain roughly 20% protein in addition to a wealth of other helpful nutrients. The frass is a by-product of BSFL rearing, consisting of a significant quantity of various nutrients. A study reported that using frass as an organic fertilizer would benefit crop growth [10]. The source, nutrient content, degree of mineralization, and storage technique of frass significantly impact its effectiveness as fertilizer [11].

Frass has many benefits in boosting soil fertility, and there has been extensive research into the mineralization and timing of N by frass generated from various organic resources [12]. However, its optimized application in soils and its effect on plants is not fully understood. Thus, this study focuses on determining nitrogen availability during the urea treatment using the frass produced by BSFL from palm oil processing wastes, namely the DC and PKE. The selected physicochemical properties of BSFL frass after being fed on DC and PKE, the suitability of BSFL frass to be used as organic fertilizer in conjunction with urea was identified. The optimal amount of BSFL frass that could be used to retain N from urea application was also studied.

2. MATERIAL AND METHODS

2.1. Preparation and Analysis of Soil Sample

The soil used in this study was Nyalau Series (*Typic Paleudults*), located in a primary forest of Universiti Putra Malaysia Bintulu Campus. The area has an elevation of 27.3 m, with an annual rainfall of 2993 mm, a mean temperature of 27 °C, and a relative humidity of about 80%. The sampling area was 50 m × 50 m where soil samples will be randomly taken from this area. The soil samples were then air-dried, ground, and sieved through a 2 mm sieve.

2.2. Initial Characterization of Soil and Frass from Decanter Cake (DC) and Palm Kernel Expeller (PKE)

Table 1 summarizes the type of initial characterization carried out on soil and frass from DC and PKE. Ammonium and nitrate are two inorganic forms of nitrogen that require attention. Important type of nitrogen needed in crop growth and development include ammonium and nitrate, booth of which are soluble in water. The acidic pH of the soil was collected from the *Typic Paleudults* (Nyalau Series). Minerals in Malaysia's naturally acidic soil are responsible for these properties [13].

Table 1 Selected physical and chemical properties of Nyalau Series (*Typic Paleudults*) and frass from decanter cake (DC) and palm kernel expeller (PKE) used in the incubation study

Properties	Soil	Frass (DC + PKE)
pH (KCl)	3.9	8.2
pH (dH_2O)	4.76	8.5
Electrical Conductivity ($\mu S\ cm^{-1}$)	n.d.	95.7
Moisture content	n.d.	24.3
Organic Matter (%)	5.03	47.5
Total Carbon (%)	2.91	26.1
Exchangeable ammonium (%)	12	1.9
Available Nitrate	5.2	1.9
Total Nitrogen (%)	0.37	1.8
Total Phosphorus (%)	110.88	4.4
Total Potassium (%)	28.83	4.5

Note: All analyses are based on dry weight; n.d.: not determined.

2.3. Incubation Set-up

The recommendation rate of N required for lettuce (test crop) was 60 kg N (130 kg ha^{-1} urea) and scaled down to per plant basis from the standard fertilizer recommendation. The BSFL frass rate was 5 t ha^{-1}, but it was scaled down to a per-plant basis. From Table 3.1, T0 represented the contribution of initial N in the soil, T1 represented available N in the soil and urea, T2 represented available N in the soil with BSFL frass, T3, and T4 represented the N in the soil with different rates of urea and BSFL frass. However, the urea and BSFL frass amounts in T3 and T4 were reduced by 50% and 25% based on standard recommendations (Table 2). The soil, urea, and frass were thoroughly mixed, after which the mixture was incubated in transparent polypropylene containers with perforated lids for good aeration. The samples were incubated at room temperature (26 °C) for 30, 60, and 90 days. Three replicates of each treatment were arranged to suit a completely randomized design (CRD) at the Soil Science Laboratory, Universiti Putra Malaysia Bintulu Sarawak Campus, Malaysia. The samples were moistened to 60% moisture content based on the soil's field capacity. The soil moisture level was maintained using distilled water when necessary.

Table 2 Selected treatments and components of the incubation of Soil, Urea, and Frass

Treatments	Components
T0	Soil only
1	Soil + Urea (4g)
T2	Soil + Frass (40g)
T3	Soil + Frass (30g) + Urea (3g)
T4	Soil + Frass (20g) + Urea (2g)

2.4. Soil Chemical Analysis Before and After Incubation

The soil samples were characterized for physical and chemical properties before and after the incubation study. Soil pH in water and potassium chloride (KCl) and electrical conductivity (EC) were measured in a 1:2.5 (soil: distilled water/KCl) using a digital pH meter and an EC meter, respectively [14]. Soil total carbon (TC) was determined using the Thermo Scientific CHNS analyser and the Total Organic Matter was calculated 1.72% of Total Carbon. The soil CEC was determined using the leaching method [5] followed by steam distillation [15]. Exchangeable cations [Ca, Mg, Sodium (Na), and Fe] were extracted with 1 M ammonium acetate (NH_4OAc), pH 7 using the leaching method [15]. Afterward, the cations were quantified using Atomic Absorption Spectrophotometry (novAA 800, analyticjena, Technology Quality Innovation, Germany). Lastly, soil inorganic N (exchangeable NH_4^+ and available NO_3^-) was determined following the method [16].

2.5. Statistical Analysis

Data were analyzed statistically using analysis of variance (ANOVA) to detect treatment effects. The means of the treatment were compared using Tukey's HSD test at $p \leq 0.05$. Statistical Analysis System (SAS) Version 9.4 was used for the statistical analysis. A normality test was performed to ensure the data obtained fit the ANOVA assumption.

3. RESULTS AND DISCUSSIONS

The selected physical and chemical properties of soil are summarised in Table 1. pH of the *Typic Paleudults* (Nyalau Series) soil extracted in water were acidic and low in electrical conductivity, field capacity, moisture content, and bulk density (Table 1). The characteristics are typical of Malaysian minerals acidic soil [17].

3.1. The Typic Paleudults (Nyalau Series) pH of Potassium Chloride and Distilled Water at Thirty Days of Incubation

Incubation from the soil affected the pH of KCl and distilled water after 30 days of incubation. The treatment without soil amendments and urea (T0) had significantly lower pH compared with the treatments with soil amendment and urea (T1, T2, T3 and T4). The soil pH levels in KCl of the soil with 100% recommended rate of frass (T2), soil with 25% recommended rate of frass and urea (T3), and soil with 50% recommended rate of frass and urea (T4) were similar and significantly higher than those T0 and T1, without the present of frass. Moreover, pH in KCl with 100% recommended rate of urea (T1) was significantly higher than T0, which is soil only. The soil pH in distilled water for T0 with soil only was significantly lower compared with the treatments with soil amendment and urea (T1, T2, T3 and T4).

The pH levels in KCl at times T2, T3, and T4 were significantly greater than those measured at times T0 and T1 (Figure 1). Whereas in distilled water T1, T2, T3 and T4 were higher than T0. The pH of the soil is one of the most important factors that can hinder mineralization. A higher pH stimulates the process of disintegration. Mineralization does, however, take place at all pH levels, and it is mediated by bacteria that are capable of thriving in those situations [18]. The application of urea to treatments (T1, T3, and T4) that undergo hydrolysis releases more OH^- ion to increase soil pH [19], At the same time, applying frass with a pH of 6.9 performs as a liming agent, which also increases soil pH [20]. The soil pH in urea microsites increased to a maximum of 8 to 9 after the application of urea [21]. It has been demonstrated that bringing the pH of a site up to that of water causes an increase in the rate of decomposition, which in turn causes an increase in the rate of N mineralization [22].

It is believed that the application of organic amendment derived from BSF can help minimize terrestrial acidification by reducing levels of aluminium and iron, hence preventing these elements from hydrolysing and creating H^+ ions [23]. The application of BSF decreased the amount of aluminium and iron that was active in the soil solution [24]. This was caused by reactions that formed organometal complexes with the hydroxyl and carboxyl functional groups of humic acids. Frass contains humic acid, which has a distinctive quality that enables it to interact with metal ions, oxides, hydroxides, and minerals, in addition to hazardous pollutants that have the ability to lower pH levels [25].

This implies that the addition of frass to tropical soils with acidic pH results in a decrease in the generation of H^+ ions. According to the findings (Figure 1), shifts in soil pH are influenced by the buffering capacity of the soil, and the pace at which urea is broken down is affected by urease activity. Both of these factors can be seen in the Figure 1.

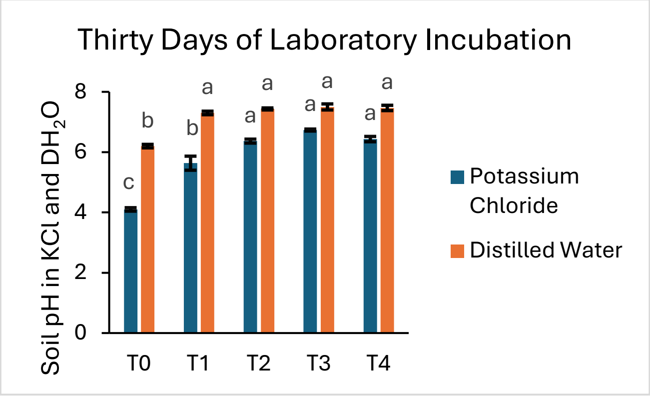

Figure 1 Soil pH of different treatments in potassium chloride and distilled water as 300 g soil only (T0) 300 g soil + 4 g urea (T1) 300 g soil + 40 g frass (T2), 300 g soil + 30 g frass (T3), and 300 g soil + 20 g frass (T4) during means of treatments using Tukey's test at $p<0.05$. The different cases of alphabets show different comparisons. Bars represent the mean values ± standard error of replications.

3.2. The Typic Paleudults (Nyalau Series) of Total Carbon and Organic Matter at Thirty Days of Incubation

The effects of treatments on soil total carbon (TC) at 30 days of incubation (DAI) are presented in Figure 2. The soil TC with 100% recommended rate of frass in T2 were significantly higher than T0, T1, T3 and T4. Soil TC with 100% recommended rate of frass in T2, soil TC with 50% recommended rate of frass and urea in T3, and soil TC with 25% recommended frass and urea in T4 was significantly higher than those T0, T1, without the present of frass. Besides, soil TOM with 100% recommended rate of frass in T2 was significantly higher than those of T0, T1, T3 and T4. Soil TOM with 100% recommended rate of frass in T3 and soil TOM with 50% recommended frass and urea in T3 were significantly higher than those of T0, T1 and T4. Soil TOM in T2, T3 and T4 with the present of frass and urea were significantly higher than T0 and T1, without the present of frass.

Figure 2 shows that treatment T2 had the highest levels of soil total carbon when compared to the other treatments (T0, T1, T3 and T4), but treatment T2 and T3 had the highest levels of soil total organic matter when compared to the other treatments (T0, T1 and T4). The relevance of soil total carbon (TC) in regulating crop yields has been well established. Applied organic amendment, particularly increases in soil total carbon (TC) [26]. The accumulation of TC can be beneficial to crop productivity in several ways: it can increase the size of the mineralizable N and P pool; it can improve soil structural properties, such as lower soil bulk density, improved aggregate stability, improved aeration, and pore connectivity; and it can ameliorate constraints, such as inappropriate soil pH and low cation exchange capacity. All these factors contribute to increased crop productivity [27]. Microbial decomposition of organic nitrogen [28] requires a substrate, which organic matter provides. Changes in the total amount of nitrogen in the soil are related to the substrate supplied from organic amendment for microbial development and their activity [29]. In addition, the microorganisms necessary for the breakdown of organic nitrogen in soils are derived from the organic matter contained in compost [30]. Urease activity is strongly intertwined with the biological activity of soils, which is directly proportional to the amount of organic matter present in addition to a variety of other elements that influence the development of microorganisms [31].

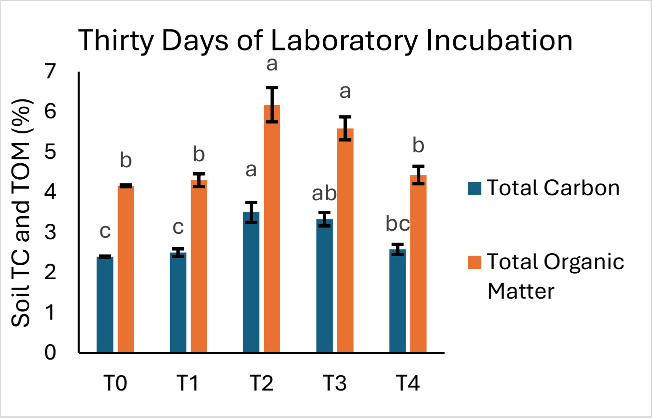

Figure 2 Soil total carbon and total organic matter of different treatments in 300 g soil only (T0) 300 g soil + 4 g urea (T1) 300 g soil + 40 g frass (T2), 300 g soil + 30 g frass (T3), and 300 g soil + 20 g frass (T4) during means of treatments using Tukey's test at $p<0.05$. The different cases of alphabets show different comparisons. Bars represent the mean values ± standard error of replications.

3.3. The *Typic Paleudults* (Nyalau Series) of Available Nitrate (NO_3^-) and Exchangeable Ammonium (NH_4^+) at Thirty Days of Incubation

The effects of treatment on soil available nitrate (NO_3^-) and exchangeable ammonium (NH_4^+) at 30 days of incubation (30 DAI) are presented in Figure 3. The soil available NO_3^- with 100% recommended rate of urea in T1 was significantly higher than T0, T2, T3, and T4. Soil available NO_3^- with 100% recommended rate of urea in T2, 50% recommended rate of frass and urea in T3, and 25% recommended rate of frass and urea in T4 were significantly similar but lower than T1 with 100% recommended of urea and higher than without frass and urea in T0. Soil available NO_3^- in T1, T2, T3, and T4 were significantly higher than T0, which is soil only. Besides, soil exchangeable NH_4^+ with 100% recommended rate of urea in T1 was significantly higher than those of T0, T2, T3 and T4. Soil NH_4^+ with 100% recommended rate of urea in T1, 100% recommended rate of frass in T2, 50% recommended rate of frass and urea in T3 and 25% recommended rate of frass and urea in T4 were significantly higher than T0, which is soil only. Soil NH_4^+ with 100% recommended rate of frass in T2, 50% recommended rate of frass and urea in T3 and 25% recommended rate of frass and urea in T4, with the present of frass were significantly similar but lower than 100% recommended rate of urea in T1 and higher than soil only in T0.

Exchangeable ammonium and nitrates play a significant role in the global nitrogen cycle, particularly the nitrogen released by organic fertilizers. When discussing the frass generated by Black Soldier Fly (BSF), it is noteworthy to mention that it possesses a substantial quantity of ammonium and nitrate (Table 1). Nevertheless, for plants to effectively utilize nitrogen, they must undergo a preliminary conversion process, creating nitrate (NO_3^-) [32]. The conversion above is facilitated by two distinct mechanisms, namely mineralization and nitrification. On the one hand, soil microorganisms have a strong affinity toward ammonium, thereby facilitating its sequestration and reducing its accessibility to plants through a phenomenon referred to as immobilization [33]. Achieving the conversion of ammonium to nitrate in a manner that optimizes its utilization by plants necessitates a delicate equilibrium.

In treatment 1 (T1), the addition of urea (4g) to the soil resulted in considerably elevated levels of soil accessible NO_3^- and NH_4^+ in comparison to the other treatments. This statement aligns with the current state of literature concerning the function of urea as a nitrogen-based fertilizer, as discussed by [34]. The hydrolysis of urea in soil is facilitated by microbial urease enzymes, resulting in the liberation of ammonium (NH_4^+) ions. These ammonium ions are subsequently subjected to nitrification, a process that converts into nitrate (NO_3^-) ions [35]. The observed increase in NH_4^+ levels after the hydrolysis of urea may have played a role in the elevated levels of NO_3^- seen in T1. As anticipated, T1 exhibited increased amounts of NH_4^+ as a result of the hydrolysis of urea. The observed elevated content of NH_4^+ in T1 aligns with the prompt formation of urea hydrolysis byproducts.

The treatments T2, T3, and T4, which incorporated the application of Black Soldier Fly frass at quantities of 40g, 30g, and 20g, respectively, exhibited significantly higher concentrations of NO_3^- in comparison to the control treatment (T0) (Figure 3). The elevation NO_3^- concentrations can be ascribed to multiple variables, one of which is the presence of organic matter. The frass when integrated into the soil, acts as a provider of organic matter (Figure 2). The presence of organic matter has been found to have a positive impact on the retention of nutrients and the activity of microorganisms [36]. This, in turn, facilitates the process of nitrification, which involves the conversion of NH_4^+ to NO_3^- [37]. The presence of frass serves as a substrate for soil microbes, hence facilitating the microbial degradation of organic matter and boosting nitrification processes. Treatments T2, T3, and T4 demonstrated increased concentrations of NH_4^+. The continuous supply of NH_4^+ to the soil can be ascribed to the emission of ammonium during the decomposition of organic matter in frass (Figure 2 and Figure 3).

The treatments T3 and T4, which involved the combination of frass and urea (Table 2), showed a moderate amount of both NO_3^- and NH_4^+ in comparison to T1 and the control treatment (T0) (Figure 3). The potential impact of the interaction between frass and urea on soil nitrogen dynamics is clear. The potential impact of frass on the rates of urea hydrolysis and nitrification processes could lead to variations in the levels of nitrogen forms and concentrations [4].

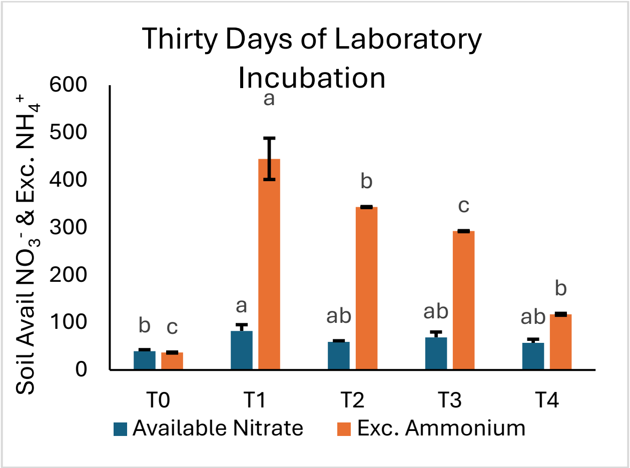

Figure 3. Soil available nitrate and exchangeable ammonium of different treatments in 300 g soil only (T0) 300 g soil + 4 g urea (T1) 300 g soil + 40 g frass (T2), 300 g soil + 30 g frass (T3), and 300 g soil + 20 g frass (T4) during means of treatments using Tukey's test at $p<0.05$. The different cases of alphabets show different comparisons. Bars represent the mean values ± standard error of replications.

3.4. The *Typic Paleudults* (Nyalau Series) of Cation Exchange Capacity (CEC) at Thirty Days of Incubation

The effects of treatments on cation exchange capacity (CEC) at 30 days of incubation (30 DAI) are presented in Figure 4. Soil CEC with 100% recommended rate of urea in T1 was significantly higher than those of T0, T2, T3 and T4. Soil CEC with 100% recommended rate of frass in T2 was significantly higher than those T0, T3 and T4 and lower than T1. Soil CEC with 50% recommended rate of frass and urea in T3 and 25% recommended rate of frass and urea in T4 were significantly similar but lower than 100% recommended rate of urea in T1 and 100% recommended rate of frass in T2 but higher than soil only in T0. Soil CEC with 100% recommended rate of urea in T1, 100% recommended rate of frass in T2, 50% recommended rate of frass and urea in T3 and 25% recommended rate of frass and urea in T4 were significantly higher compared to soil only in T0.

The cation exchange capacity (CEC) of soil increased with the incorporation of urea in T2. The degree of decomposition and the CEC of organic matter were correlated as OM decomposed to some degree in the soil, which would increase the CEC of organically dominated soils and TC content [38]. This shows that adding urea and frass in T1, T2, T3, and T4 could improve the CEC in the soil. Therefore, applying organic matter improves the soil's CEC through the presence of humic substances in the organic materials [1]. Besides, the CEC is relatable with the soil pH (Figure 1 and Figure 4) because the increasing of pH means that the concentration of H^+ cation which contributes to the acidity in the soil has been reduced, thus increasing the CEC of soil as most of the NH_4^+ could be attached to the surface of BSFL frass. T0 has low CEC as there is no addition of urea and BSFL frass.

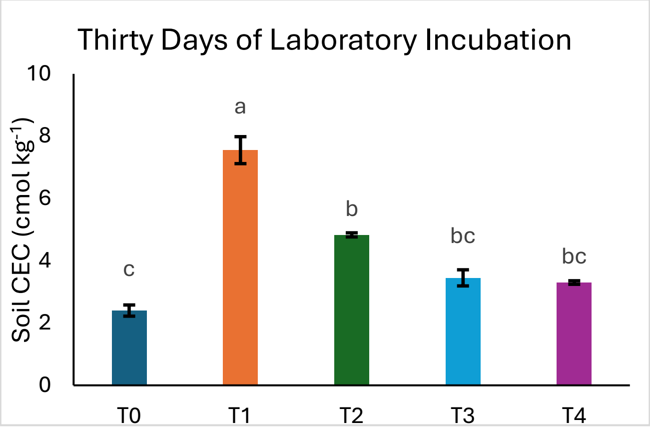

Figure 4. Soil cation exchange capacity (CEC) of different treatments in 300 g soil only (T0) 300 g soil + 4 g urea (T1) 300 g soil + 40 g frass (T2), 300 g soil + 30 g frass (T3), and 300 g soil + 20 g frass (T4) during means of treatments using Tukey's test at $p<0.05$. The different cases of alphabets show different comparisons. Bars represent the mean values ± standard error of replications.

4. CONCLUSION

The optimization application of 50% BSFL frass and 50% urea in T3 indicates that BSFL decreased by 50% compared to the existing suggestion. Additionally, the BSFL frass considerably raised soil pH. BSFL frass also significantly increase total carbon and total organic carbon. Soil CEC and pH are relatable because high pH contributes to low concentration of H^+ ion in the soil, thus increase the possibility of NH_4^+ cations to bind on the negative sites on the surface of BSFL frass. The BSFL frass also improved soil organic matter and soil total organic carbon because of the inherent content of organic matter and carbon in the BSFL frass. The higher soil total N, exchangeable NH_4^+ and available NO_3^- suggests that BSFL frass improve the retention of N in the form of NH_4^+ from urea, however, pot and field trials are essential to understand more about the ability of BSFL frass to retain N from urea and to provide essential plant nutrients.

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