Sains Malaysiana 52(3)(2023): 771-782
http://doi.org/10.17576/jsm-2023-5203-07

Importance of High Crude Fibre Insect Frass For Effective Alleviation of Ammonium (NH_4^+) Toxicity and Optimal Growth of the Short-Term Vegetable, *Amaranthus tricolor*

(Kepentingan Fras Serangga Bergentian Kasar Tinggi untuk Pengurangan Berkesan Ketoksikan Amonium (NH_4^+) dan Pertumbuhan Optimum Sayur Jangka Pendek, *Amaranthus tricolor*)

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Received: 10 August 2022/Accepted: 3 February 2023

ABSTRACT

This study focused on the efficacy of the fecal matter component from insect-derived frass containing high total crude fibre (TCF) for the effective alleviation of ammonium (NH_4^+) toxicity in short-term vegetables. Two types of insect-derived frass, bred from different feeding substrates, the black soldier fly (BSF) larvae, *Hermetia illucens*, known as the BSF frass (BSFF), and the common house cricket (CHC), *Acheta domesticus*, of both adults and nymphal instars, known as CHC frass (CHCF). The short-term vegetable, *Amaranthus tricolor*, was chosen as the test crop. A total of 200 *A. tricolor* vegetable seedlings were tested, with five experimental replicates, each with 10 *A. tricolor* seedlings set following completely randomized design, each for the different fertilizer treatments, BSFF fertilizer, CHCF fertilizer, NPK 15:15:15 fertilizer as the positive control, and no fertilizer as the negative control, pre-prepared in the form of soil–fertilizer mixtures, applied following the rate of 120 kg N ha^{-1}. Comparatively, BSFF fecal matter component recorded lower rates of decomposition of total organic matter (TOM), total organic carbon (TOC), and organic nitrogen (ON) but with a higher rate of TCF decomposition, contrary to CHCF with higher rates of decomposition of TOM, TOC, ON, and a lower rate of TCF decomposition. Additionally, the rates of ON decomposition to NH_4^+ and NH_4^+ uptake were lower for BSFF compared to CHCF. The ratio rates of TOM-TCF (22:31), TOM-ON (22:02), TOC-TCF (30:31), and TOC-ON (30:02) for BSFF recorded the highest difference, compared to CHCF with a lower difference of ratio rates; TOM-TCF (36:21), TOM-ON (36:13), TOC-TCF (38:21), and TOC-ON (38:13). The alleviation of NH_4^+ toxicity for short-term vegetables with high sensitivity toward excessive NH_4^+ uptake could only be effectively alleviated with high TCF fecal matter component of the frass, increasing decomposition toward the TCF element and reduced decomposition on the organic N element of the frass' fecal matter.

Keywords: *Acheta domesticus*; ammonium toxicity; crude fibre; *Hermetia illucens*; organic fertilizer

ABSTRAK

Kajian ini memberi tumpuan kepada keberkesanan komponen bahan tahi daripada fras terbitan serangga yang mengandungi jumlah gentian kasar tinggi (TCF) untuk mengurangkan ketoksikan amonium (NH_4^+) yang berkesan pada sayuran jangka pendek. Dua jenis fras terbitan serangga, dibiakkan daripada substrat pemakanan yang berbeza, larva lalat askar hitam (BSF), *Hermetia illucens*, dikenali sebagai BSF fras (BSFF), dan cengkerik rumah biasa (CHC), *Acheta domesticus*, kedua-dua dewasa dan instar nimfa, dikenali sebagai CHC fras (CHCF). Sayuran jangka pendek, *Amaranthus tricolor* dipilih sebagai tanaman ujian. Sebanyak 200 anak benih sayur *A. tricolor* telah diuji, dengan lima ulangan uji kaji, setiap satu dengan 10 anak benih *A. tricolor* ditetapkan mengikut reka bentuk rawak sepenuhnya, setiap satu untuk rawatan baja yang berbeza, baja BSFF, baja CHCF, baja NPK 15:15:15 sebagai kawalan positif dan tiada baja sebagai kawalan negatif, yang disediakan terlebih dahulu dalam bentuk campuran tanah-baja, digunakan mengikut kadar 120 kg N ha^{-1}. Secara perbandingan, komponen bahan tahi BSFF merekodkan kadar penguraian jumlah bahan organik (TOM), jumlah karbon organik (TOC) dan nitrogen organik (ON) yang lebih rendah tetapi dengan kadar penguraian TCF yang lebih tinggi, bertentangan dengan CHCF dengan kadar penguraian yang lebih tinggi. TOM, TOC, ON dan kadar penguraian TCF yang lebih rendah. Selain itu, kadar penguraian ON kepada pengambilan NH_4^+ dan NH_4^+ adalah lebih rendah untuk BSFF berbanding CHCF. Kadar nisbah TOM-TCF (22:31), TOM-ON (22:02), TOC-TCF (30:31) dan TOC-ON (30:02) untuk BSFF mencatatkan perbezaan tertinggi, berbanding CHCF dengan perbezaan kadar nisbah yang lebih rendah; TOM-TCF (36:21), TOM-ON (36:13), TOC-TCF (38:21) dan TOC-ON (38:13). Pengurangan ketoksikan NH_4^+ untuk sayuran jangka pendek dengan kepekaan yang tinggi terhadap pengambilan NH_4^+ yang berlebihan hanya boleh dikurangkan dengan berkesan dengan komponen bahan tahi TCF yang tinggi dalam fras, meningkatkan penguraian ke arah unsur TCF dan mengurangkan penguraian pada unsur N organik fras tahi.

Kata kunci: *Acheta domesticus*; baja organik; gentian kasar; *Hermetia illucens;* ketoksikan amonium

INTRODUCTION

Insect species currently mass-farmed in Malaysia are the common house crickets (CHC) (*Acheta domesticus*), to produce biomass rich in proteins and lipids as aquafeed raw materials. The farming of CHC, similar to black soldier fly (BSF), generated by-product wastes, known as CHC frass (CHCF), composed of the dead CHC individuals of various nymphal instar stages, the cuticular wastes from nymphal instars' ecdysis processes, and the fecal matter from the feeding on the high-protein broiler starter crumble chicks' feeds (BSCCFs), to produce rapid growth of farmed CHCs. The effect of CHCF as potential fertilizer has never been evaluated for its efficacy on plant growth. The implementation of BSF (*Hermetia illucens*) larvae (BSFL) for the decomposition of agricultural solid wastes (ASWs) in Malaysia is still at the inception level, with no current nationwide practical applications for large-scale decompositions of ASWs in landfills but rather only limited to private companies with capacities to mass-rear BSFL to producing BSFL for poultry raw feedstocks. However, at the global level, the applications of BSFL for the management of successful decompositions of organic solid wastes showed numerous successes (Čičková et al. 2015; Gold et al. 2018). Apart from the successful implementations of BSFL in decomposing organic solid wastes, the processing residue, also known as the BSF frass (BSFF) produced after the complete decomposition of organic solid wastes also exhibited excellent properties as novel insect-based organic fertilizer (Beesigamukama et al. 2020) and showing positive synergisms with synthetic chemical fertilizers (Tanga et al. 2021).

Previous achievements of BSFF fertilizers were attributed to non-ammonium (NH_4^+) sensitive crops. However, most NH_4^+-sensitive short-term crops were exposed to higher risks of NH_4^+ toxicity, since the powdered fecal matter component of insect-derived frass was predominantly composed of NH_4^+ as the sole inorganic-N (Kagata & Ohgushi 2012, 2011), dependent on the types of organic wastes provided to the developing BSF larvae (Salomone et al. 2017). NH_4^+ toxicity resulted in substantial suppression of NH_4^+ sensitive crop growths (Alattar, Alattar & Popa 2016; Liu et al. 2009). Cuticular wastes derived from dead BSF larval individuals (Devic 2016) were composed of the crystalline insoluble chitin polysaccharide, which was inconsistent in volume and had low inorganic-N bioavailability and low decomposition rates, limiting its effective utilizations (Pillai, Paul & Sharma 2009; Ravi Kumar 2000). NH_4^+ toxicity risks were further escalated by employing the accessible topsoil with clay mineral characteristic of low affinity for NH_4^+ adsorption (Gärttling, Kirchner & Schulz 2020; Kagata & Ohgushi 2012; Ma et al. 2018).

Based on these arguments, this study was conducted to assess in-depth the importance of the characteristics of the fecal matter component of both BSFF and CHCF, derived from two different insect species, BSF (*H. illucens*) and CHC (*A. domesticus*), respectively, farmed from two distinct farming system of different feed types, involving soil with clay mineral of low NH_4^+ adsorption capacity (kaolinite).

MATERIALS AND METHODS

STUDY SITES

The study was conducted from August to November 2021 at the School of Biological Sciences (SBS) Plant House, L14 Building, Universiti Sains Malaysia (USM), Penang Island, Malaysia (5°21′26.4″ N and 100°17′39.9″ E), where the soil was classified as acidic granitic soil, with medium- to coarse-grained porphyritic muscovite–

TABLE 1. Proximate chemical attributes of top-soil used in the pot-or-polybag culture study collected from USM Plant House L14-Building

Parameters	Measurements
pH	6.40
Fine sand (%)	38.90
Coarse sand (%)	26.90
Clay (%)	28.10
Silt (%)	6.10
Total Nitrogen (N) (% w/w)	0.07
Organic Nitrogen (mg kg^{-1})	1680.00
Exchangeable Ammonium NH_4^+ (mg kg^{-1})	9.10
Exchangeable Nitrite NO_2^- (mg kg^{-1})	0.01
Exchangeable Nitrate NO_3^- (mg kg^{-1})	0.03
Total Potassium (K) (mg kg^{-1})	21.50
Exchangeable Potassium (K^+) (mg kg^{-1})	15.50
Exchangeable Calcium (Ca^{2+}) (mg kg^{-1})	34.50
Exchangeable Magnesium (Mg^{2+}) (mg kg^{-1})	4.00
CEC (meq/100g)	5.30
Electrical Conductivity (EC) (µS/cm)	72.80
C/N Ratio	5.70
Total Organic Carbon (TOC) (% w/w)	0.40
Total Organic Matter (TOM) (% w/w)	8.90
Total Crude Fibre (TCF) (% w/w)	2.10

biotite granite with microcline (Ahmad, Yahaya & Farooqi 2006; Kong 1994; Trakoonyingcharoen et al. 2006). Topsoil (0-20 cm depths) was used as the potting medium for this study. Table 1 summarizes the soil type (based on the USDA soil texture classification) and other related physicochemical characteristics.

CROP ESTABLISHMENT AND AGRONOMIC PRACTICES

Gathered topsoils (0-20 cm depths) around SBS USM Plant House, L14 Building, were first temperature-treated at 60 °C for 30 min to eliminate all possible soil pathogenic microorganisms while maintaining the probability for the survival of topsoil beneficial denitrifying microbial community. The temperature-treated soils were further sieved via a 1.0 mm stainless steel sieve. The soil physicochemical characteristics were analyzed at the beginning of the experiment (Table 1). BSFF, CHCF, and the slow-release, granulated NPK 15:15:15 inorganic compound fertilizer as a positive control were used in this study. BSFF was supplied by a private company specifically mass-reared BSFL for decomposing supplied ASWs, consisting of several rotting or decomposing plant-based materials of various tropical plant species, comprising leafy vegetables, vegetable fruits, and fruits. CHC of both developing nymphal and adult stages were equally fed with commercially formulated high-protein BSCCFs. CHCF was supplied by a private company specifically producing fresh adult CHC for aquaculture raw materials, where CHCF was considered to be a by-product of CHC (*A. domesticus*)

TABLE 2. Proximate chemical attributes for all fertilizers evaluated in this study; NPK 15:15:15 (positive control, Black Soldier Fly frass (BSFF), and Common House Cricket frass (CHCF). Abbreviations: Ex.: Exchangeable, TOC: Total Organic Carbon, TOM: Total Organic Matter, TCF: Total Crude Fibre

Parameters	BSFF	CHCF	NPK 15:15:15
pH	9.10	8.30	5.90
Total N (% w/w)	3.05	4.43	16.23
Organic N (mg kg^{-1})	1980.00	2340.00	2600.00
Ex. NH_4^+ (mg kg^{-1})	4003.30	10293.70	2476.60
Ex. NO_2^- (mg kg^{-1})	0.04	0.01	0.02
Ex. NO_3^- (mg kg^{-1})	0.10	0.03	0.04
Total K (mg kg^{-1})	66148.99	16537.84	130068.03
Total Ca (mg kg^{-1})	3451.78	3065.12	2455.94
Total Mg (mg kg^{-1})	2555.20	1971.83	1664.72
CEC (meq/100g)	32.90	20.00	13.10
EC (μS/cm)	7940.00	6580.00	3720.00
C/N Ratio	10.20	7.40	0.10
Total Ash (% w/w)	22.90	10.40	57.50
TOC (% w/w)	25.30	29.00	1.60
TOM (% w/w)	79.70	90.50	41.20
TCF (% w/w)	22.20	2.80	0.00 (Not detected)

farming. The fertilizers' proximate chemical characteristics were analyzed by certified and accredited laboratories in Malaysia, following the AOAC standards (Latimer 2016). Table 2 shows the results of laboratory proximate chemical analyses.

Both the insect-based frasses and the NPK 15:15:15 inorganic compound fertilizers were applied during the transplanting. The Malaysian Agricultural Research and Development Institute had established specific recommendations for *Amaranthus* spp. (Chinese Spinach) fertilizer rate application of 60 kg N ha^{-1}. However, since this study involved the use of topsoil with limited N-source from the subsoil layers (Kundu, Ladha & Guzman 1996), the N application rate was doubled following 120 kg N ha^{-1}. NPK 15:15:15 inorganic compound fertilizer was applied following the recommended N rate (740 kg ha^{-1}, i.e., 120 kg N ha^{-1}), followed by BSFF and CHCF fertilizers application rates at 3.934 t ha^{-1} and at 2.708 t ha^{-1} (120 kg N ha^{-1}), respectively. Fertilizers were applied via soil–fertilizer premixed mixtures to produce uniform fertilizer distribution to all *A. tricolor* seedlings, following the soil dilution method and put into a standard polybag (35 cm × 35 cm × 25 cm) with a maximum capacity of ±5 kg. Soil moisture levels were constantly maintained with the application of 130 mL (Mesgaran et al. 2021) of deionized water for each of the *A. tricolor* seedlings twice daily employing veterinary-grade stain less steel syringes (80 mL maximum fluid holding volume; 10 mm diameter hypodermic stainless-steel needle).

EXPERIMENTAL DESIGN

Amaranthus tricolor, a species of short-term annual tropical leafy vegetable crop, available worldwide, was used as a test vegetable crop to assess the fertilization efficacies of both BSFF and CHCF fertilizers in this study. Completely randomized design with five experimental replicates for each treatment (BSFF and CHCF fertilizers) and NPK 15:15:15 fertilizer as a positive control was employed, including a negative control (without fertilizer application), where one experimental replicate involved 10 *A. tricolor* seedlings, with a total of 200 seedlings used in this study. *Amaranthus tricolor* seedlings were transplanted approximately after two weeks following successful germination and sown started by mid-August 2021 and harvested by mid-October 2021 after approximately 8 weeks (±56 days) after the transplanting process. The seedlings were chosen for the process of transplantation into respective polybags of pre-prepared soil–fertilizer mixtures based on the minimum achieved homogenous standardized heights (mm) of approximately ±20 mm. Five replicates (one polybag = one replicate) were established for each of the premixed soil–fertilizer mixtures, including the negative control treatment, with a total of 20 polybags.

POSTHARVEST SOIL ANALYSIS

Postharvest soils at the closest level to the root system for all treatments were retrieved from one polybag containing seedlings with the highest growths for all measured shoot systems. Postharvest soils were subjected to proximate chemical analyses, including soil potential of hydrogen (pH); total N (%); inorganic-N exchangeable concentrations (NH_4^+, NO_2^-, and NO_3^-, mg kg^{-1}); organic N (urea) (mg kg^{-1}); total K (mg kg^{-1}); exchangeable K^+, Ca^{2+}, and Mg^{2+} (mg kg^{-1}); cation exchange capacity (CEC) (meq/100 g); electrical conductivity (EC) (μS/cm); C:N ratio; total crude fibre (TCF, % w/w); total organic carbon (TOC, % w/w); and total organic matter (TOM, % w/w). All listed proximate chemical analyses for the postharvest soils were run by several certified and accredited laboratories in Malaysia (UNIPEQ Pvt. Ltd., Allied Chemists Laboratory Pvt. Ltd., Bio-Synergy Laboratories Pvt. Ltd., ALS Technichem Pvt. Ltd., and FGV Analytical Laboratory Pvt. Ltd.), according to direct and modified 'In-House' analytical methods based on AOAC standards (Latimer 2016).

DATA COLLECTION

Data were collected at a 7-day interval starting from the 7th to the 56th days (approximately 8 weeks) after transplanting. Shoot system growth measurements included the height (mm), leaf length (mm), leaf width (mm), stem diameter (mm), shoot diameter (mm), root length (mm), root width (mm), and the number of leaves. Height (mm) was first measured using a thick flexible white thread as a medium for measurement, marked with a red permanent marker according to the similar fixed position of the base of the stem nearest to the soil surface and to the uppermost tip of the highest shoot. The base of each *A. tricolor* lower stem nearest to the soil surface was also marked with a red permanent marker to maintain a fixed similar initial measuring position. The marked flexible white thread was readapted or remeasured to a 600 mm digital caliper (±0.01 resolution). Leaf length (mm), leaf width (mm), stem diameter (mm), shoot diameter (mm), root length (mm), and root width (mm)) were measured using a 600 mm digital caliper (±0.01 resolution). Five initial developed leaves were initially marked and chosen per vegetable per polybag, consistently and gradually measured at a 7-day interval, and averaged for the final growth measurements.

Leaf length (mm) was measured based on the midline of the leaf's midrib, starting from the closest base of the petiole to the axial tip or apex of each of the measured leaves. Leaf width (mm) measurement was standardized based on a horizontal line position that was proportionally perpendicular to the midpoint of the vertical midrib. Stem was differentiated from shoot based on the last apical petioles extending from the shoot. Stem diameter (mm) was measured based on the midpoint between the lowest shoot with several older petioles of the shoot and the uppermost root line (the distinction between the greenish-whitish colour of the stem and the total whitish colour of the root). Shoot diameter (mm) was measured based on the midline with respect to the vertical length of the shoot from the lowest extending petioles to the uppermost tip with newly developed petioles. For root length (mm) and root width (mm), the main vertical primary root was measured, separated from the base of the stem with defined total whitish colour compared to the base of the stem with a mixture of greenish-whitish in colour. Root length (mm) was measured from the lowest horizontal greenish-whitish line of the stem up to the vertical whitish end tip of the root. Root width (mm) was measured based on the midpoint perpendicular to the measured vertical root length (mm). The postharvest biomass measurements included the overall plant fresh and dry weights (g), fresh and dry stem weights (g), fresh and dry shoot weights (g), fresh and dry leaf weights (g), and fresh and dry root weights (g). Each of the listed *A. tricolor* parts was dried in a drying oven at 70 °C for 48 h, following the weighing of the respective resulting dry weights (g). Data were collected from all 10 *A. tricolor* vegetables per polybag (replicate) per fertilizer type for both the shoot system growth measurements and the postharvest biomass measurements.

DATA ANALYSIS

The recorded raw data for both shoot systems and postharvest biomasses of fresh and dry weights (g) were independent of different seedlings, within the same polybag and among different polybags of similar fertilizer treatment applied. One-way Welch's analysis of variance (ANOVA) with bootstrapping was run for all of the above-ground shoot systems following Games-Howell post hoc tests since all above-ground shoot systems data showed heteroscedastic variances data patterns. One-way ANOVA was run for root lengths (mm), root widths (mm), and all the fresh and dry postharvest weights (g) following Tukey's HSD post hoc tests to determine the significant differences among the applied fertilizers as the source of variations. All data were analyzed using IBM SPSS Statistics Software version 26.0.0.0 statistical software of 64-bit edition.

RESULTS AND DISCUSSION

CHCF fertilizer-treated *Amaranthus tricolor* vegetables displayed significantly reduced growth parameters for both shoot systems and postharvest biomass fresh and dry weights, compared to BSFF fertilizer treatment (Table 3).

TABLE 3. Mean ± S.E. Mean of *Amaranthus tricolor* shoot system growth parameters (mm), fresh and dry post-harvest biomass (g) according to the tested fertilizers (NPK 15:15:15, BSFF, and CHCF), including control treatment

Variables	BSFF	CHCF	NPK 15:15:15	Control
Hgt. (mm)	$190.74 \pm 18.41_a$	$117.32 \pm 11.33_b$	$159.90 \pm 15.33_a$	$95.37 \pm 9.21_b$
Shoot Dia. (mm)	$3.19 \pm 0.33_a$	$1.96 \pm 0.20_b$	$2.66 \pm 0.27_a$	$1.59 \pm 0.17_b$
Stem Dia. (mm)	$3.75 \pm 0.43_a$	$2.31 \pm 0.26_b$	$3.13 \pm 0.36_a$	$1.88 \pm 0.21_b$
Leaf Length (mm)	$53.95 \pm 3.94_a$	$33.18 \pm 2.42_b$	$44.95 \pm 3.28_a$	$26.97 \pm 1.97_b$
Leaf Width (mm)	$29.89 \pm 3.16_a$	$18.38 \pm 1.95_b$	$24.90 \pm 2.63_a$	$14.94 \pm 1.58_b$
Root Length (mm)	$90.48 \pm 5.87_a$	$55.19 \pm 3.48_b$	$75.10 \pm 5.07_a$	$45.24 \pm 2.98_b$
Root Width (mm)	$9.30 \pm 0.86_a$	$5.68 \pm 0.52_b$	$7.72 \pm 0.71_a$	$4.65 \pm 0.43_b$
No. of leaves	$7.80 \pm 0.34_a$	$6.66 \pm 0.24_b$	$7.15 \pm 0.32_a$	$6.46 \pm 0.24_b$
Total Fresh Wt. (g)	$25.56 \pm 3.11_a$	$15.72 \pm 1.92_b$	$21.29 \pm 2.59_a$	$12.79 \pm 1.56_b$
Total Dry Wt. (g)	$9.94 \pm 1.21_a$	$4.09 \pm 0.50_b$	$5.77 \pm 0.70_a$	$2.81 \pm 0.34_b$
Shoot Fresh Wt. (g)	$11.11 \pm 1.46_a$	$6.83 \pm 0.90_b$	$9.26 \pm 1.22_a$	$5.55 \pm 0.74_b$
Shoot Dry Wt. (g)	$4.32 \pm 0.57_a$	$1.77 \pm 0.24_b$	$2.51 \pm 0.33_a$	$1.22 \pm 0.16_b$
Stem Fresh Wt. (g)	$11.30 \pm 1.02_a$	$6.95 \pm 0.64_b$	$9.41 \pm 0.85_a$	$5.65 \pm 0.52_b$
Stem Dry Wt. (g)	$4.39 \pm 0.40_a$	$1.81 \pm 0.17_b$	$2.56 \pm 0.23_a$	$1.25 \pm 0.11_b$
Leaf Fresh Wt. (g)	$7.10 \pm 1.05_a$	$4.36 \pm 0.65_b$	$5.91 \pm 0.88_a$	$3.55 \pm 0.53_b$
Leaf Dry Wt. (g)	$2.77 \pm 0.41_a$	$1.14 \pm 0.17_b$	$1.60 \pm 0.24_a$	$0.78 \pm 0.12_b$
Root Fresh Wt. (g)	$3.18 \pm 0.69_a$	$1.95 \pm 0.43_b$	$2.64 \pm 0.58_a$	$1.59 \pm 0.35_b$
Root Dry Wt. (g)	$1.24 \pm 0.27_a$	$0.51 \pm 0.11_b$	$0.72 \pm 0.16_a$	$0.35 \pm 0.08_b$

Hgt.: Height, Dia.: Diameter, Wt.: Weights

These observed growth parameters' results, as well as based on the subsequent analyses, are to be attributed mainly to the effect of NH_4^+ toxicity, since NH_4^+ was the only predominant inorganic, plant-available N-form present and recorded in all postharvest soils treated with the respective applied and tested fertilizers of BSFF, CHCF, and NPK 15:15:15, including the control treatment. The lowest NH_4^+ concentration (79.80 mg kg^{-1}) in the postharvest soil of CHCF fertilizer treatment, contradicted the highest NH_4^+ concentration in CHCF fertilizer (10293.70 mg kg^{-1}) and the highest postharvest soil CEC level (7.20 meq/100 g). It was not possible for the potential leaching of NH_4^+ out of the soil rhizosphere, reflecting the increased uptake of NH_4^+. Postharvest soil's low nitrate (NO_3^-) concentration (0.01 mg kg^{-1}), a relatively similar concentration as the initial topsoil (0.03 mg kg^{-1}) showed no possible nitrification process.

Expectedly, if nitrification had occurred, the concentration of NO_3^- could be comparably higher than other postharvest soils with the introduced fertilizer-originated NH_4^+ and subsequent oxidation of NH_4^+, as well as with increased uptake of NH_4^+ compared to NO_3^-, or at least to be at the detectable level at 0.10 mg kg^{-1} as opposed in the fertilizer form. The postharvest soils treated with both BSFF and NPK 15:15:15 fertilizers, on the other hand, recorded lower CEC readings (7.00 meq/100 g and 6.60 meq/100 g), with moderate and low remaining NH_4^+ concentrations (NPK 15:15:15: 2476.60 mg kg^{-1} and BSFF: 4003.30 mg kg^{-1}) (697.40 and 277.90 mg kg^{-1}), respectively, for BSFF and NPK 15:15:15, and exhibited significantly positive growths. Similarly, both postharvest soils of BSFF and NPK 15:15:15-treated fertilizers recorded very low NO_3^- concentrations (equally at 0.02 mg kg^{-1}) (Table 4).

TABLE 4. Proximate chemical attributes of post-harvest soils after the harvesting of *Amaranthus tricolor* and the implementations of all tested fertilizers (BSFF, CHCF, and NPK 15:15:15), including control treatment

Parameters	BSFF	CHCF	NPK 15:15:15	Control
pH	9.20	6.80	7.70	6.20
Total N (% w/w)	0.63	0.27	0.33	0.08
Organic N (mg kg^{-1})	1870.00	1560.00	1260.00	1890.00
Ex. NH_4^+ (mg kg^{-1})	697.40	79.80	277.90	15.70
Ex. NO_2^- (mg kg^{-1})	0.01	0.01	0.01	0.01
Ex. NO_3^- (mg kg^{-1})	0.02	0.01	0.02	0.03
Total K (mg kg^{-1})	6960.00	1160.00	2115.00	950.00
Ex. K^+ (mg kg^{-1})	590.00	17.50	140.00	14.50
Ex. Ca^{2+} (mg kg^{-1})	100.00	67.00	51.50	35.00
Ex. Mg^{2+} (mg kg^{-1})	73.00	55.50	17.50	5.50
CEC (meq/100g)	7.00	7.20	6.60	6.30
EC (µS/cm)	1646.00	149.00	279.00	54.70
C/N Ratio	9.70	6.70	2.40	7.50
TOC (% w/w)	6.10	1.80	0.80	0.60
TOM (% w/w)	35.60	9.90	7.70	8.70
TCF (% w/w)	4.90	1.30	2.10	2.50

Ex.: Exchangeable; TOC: Total Organic Carbon; TOM: Total Organic Matter; TCF: Total Crude Fibre

Very low NO_3^- levels of initial topsoil and all cases of postharvest soils including the negative control (no fertilizer) show the possibility of the absence of nitrification process, where topsoil with relatively high clay portion (>25% clay) has the potential for higher denitrification rates, associated with an increased denitrifying microbial community (Jha et al. 2017; Li et al. 2021; Qasim et al. 2022; Soana et al. 2022), maintaining NH_4^+ as the only predominant inorganic-N available for plant uptake.

The rate of pH increased (%) observed in BSFF fertilizer treatment of the postharvest soil condition was at 43.75%, compared to CHCF fertilizer at 6.25% of pH increment. In terms of the ratio of BSF to CHCF of pH increment, it was at the ratio of 18:03, or approximately six times higher for BSFF compared to CHCF. High TCF of BSFF fertilizer induced for 58.06% (TCF: pH = 31:18) of pH increased in relation to TCF decomposition, compared to CHCF fertilizer with only 14.29% (TCF: pH = 21:03) of pH increased associated with the decomposition of lower TCF. The rate of TOM decomposition is lower for BSFF (55.33%), followed by lower TOC decomposition (75.89%) and organic nitrogen (ON) (5.56%) but with higher TCF decomposition (77.93%), compared to CHCF with a higher TOM decomposition rate (89.06%), followed by higher TOC decomposition (93.79%) ON (33.33%) but of lower TCF degradation (53.57%). However, despite the lower TCF degradation of CHCF fertilizer, it was higher than the degradation of ON, with the ratio of TOC-ON (38:13), lower than TOC-TCF (38:21). BSFF fertilizer's rate of ON conversion to NH_4^+ (50.54%) and rate of NH_4^+ uptake rate (82.58%) were both lower compared to CHCF fertilizer with the rate of ON conversion to NH_4^+ at 77.27%, and the rate of NH_4^+ uptake at 99.22%.

Additionally, comparative ratio rates between the decomposition of TOM and TOC in relation to both TCF and ON for CHCF fertilizers showed that the decompositions' ratio between TOM and TOC with TCF was slightly higher but comparable (TOM: TCF = 36:21; TOC: TCF = 38:21) with ON (TOM: ON = 36:13; TOC: ON = 38:13), compared to BSFF fertilizer with significant wide ratio difference for the decompositions of TOM and TOC with TCF (TOM: TCF = 22:31; TOC: TCF = 30:31) compared to ON (TOM: ON = 22:02; TOC: ON = 30:02) (Table 5).

TABLE 5. The differential rates (%) and ratio rates of pH increments, TOM, TOC, TCF, and ON decompositions, TCF-pH increment, ON-NH_4^+ conversion, and NH_4^+ uptake of BSFF and CHCF insect-based frass fertilizers, with comparison of Control Treatment (CTRL)

Parameters	BSFF	CHCF	CTRL	BSFF: CHCF	BSFF: CTRL	CHCF: CTRL
pH increment (%)	43.75%	6.25%	3.13%			
pH increment ratio				18:03	18:01	03:01
TOM decomp. (%)	55.33%	89.06%	2.25%			
TOM decomp. ratio				22:36	22:01	36:01
TOC decomp. (%)	75.89%	93.79%	50.00%			
TOC decomp. ratio				30:38	30:20	38:20
TCF decomp. (%)	77.93%	53.57%	19.05%			
TCF decomp. ratio				31:21	31:08	21:08
ON decomp. (%)	5.56%	33.33%	12.50%			
ON decomp. ratio				02:13	02:05	13:05
ON-NH_4^+ (%)	50.54%	77.27%	99.46%			
ON-NH_4^+ ratio				20:31	20:40	31:40
NH_4^+uptake (%)	82.58%	99.22%	72.53%			
NH_4^+ uptake ratio				33:40	33:29	40:29
TCF: pH	31:18	21:03	08:01			
ON-NH_4^+: NH_4^+ uptake	20:33	31:40	40:29			
TOM-ON ratio (TOM: ON)	22:02	36:13	01:05			
TOM-TCF ratio (TOM: TCF)	22:31	36:21	20:08			
TOC-ON ratio (TOC: ON)	30:02	38:13	20:05			
TOC-TCF ratio (TOC: TCF)	30:31	38:21	20:08			

TOM-TCF with respect to TOM-ON for CHCF fertilizer provided 1.62 times microbial attack and decomposition protection, of very low protection compared to BSFF fertilizer with 15.5 times more protection provided by TOM-TCF with respect to TOM-ON decomposition. Similarly, for TOC-TCF and TOC-ON, BSFF fertilizer TOC-TCF provided 15.5 times higher protection against the decomposition of TOC-ON, and CHCF provided 1.62 times lower protection for TOC-ON decomposition. From this view, TOM-TCF and TOC-TCF complexes for BSFF fertilizer provided the highest protection against microbial attack and decomposition from the complexes of TOM-ON and TOC-ON compared to CHCF fertilizer with a comparable difference, provided ineffective protection against microbial attack and decomposition of ON, hence increased release and uptake of NH_4^+, leading to NH_4^+ toxicity. From these results, it could be firmly postulated that the higher TCF as the characteristic of BSFF fertilizer reduced microbial attack and decomposition of ON, hence reducing the risks of increased release and uptake of NH_4^+, as the essential inherent mechanism toward the natural alleviation of NH_4^+ toxicity.

Interestingly, postharvest soil of control treatment without external N supply recorded a higher ON decomposition rate at 12.50%, compared to postharvest soil following BSFF fertilization at 5.56%, additionally proving the critical importance of high TCF supplied from fertilizer, where soil-originated TCF (SOTCF) was not optimally adequate in reducing the rate of ON decomposition, even in the form of soil–fertilizer premix condition, and as it was initially postulated that without external N supply, ON decomposition rate would be reduced. ON decomposition rate reduction was only achievable via the externally supplied high fertilizer-originated TCF, increasing TCF decomposition to be higher than that of ON decomposition rate. Most studies proved that organic fertilizer bolstered the soil microbial community for decomposing soil organic matter (SOM) decomposition, and this study referred to TCF as one of the SOM components (Nakhro & Dkhar 2010; Wu et al. 2020).

Control treatment without externally introduced TCF recorded 19.05% of the SOTCF decomposition rate (Table 5), showing that NPK 15:15:15 fertilizer inhibited SOTCF decomposition. This was in line with other studies where mineral inorganic fertilizers decreased soil microbial metabolic quotient (Rao et al. 2021), as well as reduced soil microbial diversity and biomass (Bebber & Richards 2022; Francioli et al. 2016; Geisseler & Scow 2014). From this view, NPK 15:15:15 fertilizer had limited capacity for increased soil alkalinization, compared to BSFF fertilizer with undisrupted microbially mediated TCF decomposition and increased deprotonation of soil rhizosphere.

Although powdered BSFF fertilizer recorded a higher rate of ON decomposition to NH_4^+ compared to the slow-release, granulated NPK 15:15:15 fertilizer (NPK 15:15:15: BSFF = 2:20); interestingly, BSFF fertilizer also recorded lower uptake of NH_4^+ (NPK 15:15:15: BSFF = 36:33). Powdered CHCF fertilizer recorded both the highest rate of ON decomposition to NH_4^+ (NPK 15:15:15: CHCF = 02:31) and NH_4^+ uptake (NPK 15:15:15: CHCF = 36:40) (Table 6). The significance of high TCF in BSFF fertilizer for the effective alleviation of NH_4^+ toxicity could be observed in the higher rate of soil rhizosphere's alkalinization, inducing a slower rate of NH_4^+ uptake, even with prior condition of an increased rate of ON decomposition to NH_4^+.

There was no observable role of SOTCF to reduce NH_4^+ uptake rate following the application of the slow-release, granulated NPK 15:15:15 fertilizer treatment with 0.00% undetectable TCF, where the initial soil TCF and postharvest soil TCF difference showed 0.00% rate of decomposition, different from BSFF fertilizer with the highest recorded TCF (BSFF TCF = 22.20%; postharvest TCF = 4.90%) with 77.93% TCF decomposition, inducing up to 19.48% of soil rhizosphere's alkalinization (Yan et al. 1996) compared to NPK 15:15:15 (BSFF; pH = 9.20, NPK 15:151:15; pH = 7.70) shifting rhizosphere's N equilibrium of available NH_4^+ toward soil-bound ammonia (NH_3) (Mohammed-Nour, Al-Sewailem & El-Naggar 2019; van Rooyen et al. 2021), effectively reduced NH_4^+ uptake without the dependency on slow-release granular mechanism.

The rate of total K reduction for BSFF fertilizer was lower (89.48%) compared to CHCF fertilizer (92.99%), with the ratio rate of BSFF to CHCF of 36:37, since CHCF fertilizer recorded total K concentration approximately 4 times lower than BSFF fertilizer (BSFF: 66.148.99 mg kg^{-1}; CHCF: 16537.84 mg kg^{-1}). Additionally, with the lower total K, the rate of conversion of total K to exchangeable K^+ was higher for CHCF fertilizer (98.49%) when compared to BSFF fertilizer at 91.52%, with the ratio rate of BSFF to CHCF (37:39). Higher rate of release of exchangeable K^+ of the total K in the rhizosphere of *A. tricolor* treated with CHCF fertilizer could further induced higher affinity toward decreased adsorption and increased uptake of NH_4^+ (Roosta & Schjoerring 2008a; Zhang et al. 2022), evidently as the ratio remaining NH_4^+

TABLE 6. The differential rates (%) and ratio rates of pH increments, TOM, TOC, TCF, and ON decompositions, TCF-pH increment, ON-NH_4^+ conversion, and NH_4^+ uptake of BSFF and CHCF insect-based frass fertilizers, with comparison of slow-released NPK 15:15:15

Parameters	BSFF	CHCF	NPK	NPK: BSFF	NPK: CHCF
pH increment (%)	43.75%	6.25%	20.31%		
pH increment ratio				08:18	08:03
TOM decomp. (%)	55.33%	89.06%	81.31%		
TOM decomp. ratio				33:22	33:36
TOC decomp. (%)	75.89%	93.79%	50.00%		
TOC decomp. ratio				20:30	20:38
TCF decomp. (%)	77.93%	53.57%	0.00%		
TCF decomp. ratio				00:31	00:21
ON decomp. (%)	5.56%	33.33%	51.54%		
ON decomp. ratio				21:02	21:13
ON-NH_4^+ (%)	50.54%	77.27%	4.75%		
ON-NH_4^+ ratio				02:20	02:31
NH_4^+ uptake (%)	82.58%	99.22%	88.78%		
NH_4^+ uptake ratio				36:33	36:40
TCF: pH	31:18	21:03	00:08		
ON-NH_4^+: NH_4^+ uptake	20:33	31:40	02:36		
TOM-ON ratio (TOM: ON)	22:02	36:13	33:21		
TOM-TCF ratio (TOM: TCF)	22:31	36:21	33:00		
TOC-ON ratio (TOC: ON)	30:02	38:13	20:21		
TOC-TCF ratio (TOC: TCF)	30:31	38:21	20:00		

between BSFF to CHCF fertilizers in the postharvest soils was at 279:32. Comparison to the concentrations of K^+ to NH_4^+ of both fertilizers in the postharvest soils showed similar pattern of increased tendencies toward the uptake of K^+ over NH_4^+ (BSFF; 236:279, CHCF; 7:32), with 1.2 times of increased uptake of K^+ over NH_4^+ for BSFF, and 4.6 times of increased uptake of K^+ over NH_4^+, where with higher or increased uptake pattern of K^+ over NH_4^+ observed for CHCF fertilizer treatment to *A. tricolor* seedlings did not effectively alleviate the effect of NH_4^+ toxicity (Dlamini et al. 2020; Esteban et al. 2016).

The ineffective alleviation of NH_4^+ toxicity could be attributed to the physiology of *A. tricolor* vegetables, as most of the vegetables of the genus *Amaranthus* are NO_3^- tolerant, with higher sensitivity toward NH_4^+ of marked reduced growths and increased physiological stresses when NH_4^+ was applied as the sole inorganic-N (Munene et al. 2017) since most other plants with higher tolerant toward increased uptake of NH_4^+ recorded alleviation of NH_4^+ toxicity following increased uptake of K^+ (Esteban et al. 2016). CHCF fertilizer with the highest ON decomposition to NH_4^+ and NH_4^+ uptake rates with OM high in ON and low TCF induced antagonistic uptake competition between NH_4^+ and K^+, resulting in increased primary uptake of NH_4^+ and decreased uptake of K^+. Subsequently, after NH_4^+ toxicity effects were exhibited, secondary increased K^+ uptake took place, due to a physiological compensation process to alleviate the existing NH_4^+ toxicity (Esteban et al. 2016; Roosta & Schjoerring 2008b; Weng et al. 2020).

Conclusion

BSFF fertilizer with the characteristics of OM high in TCF produced lower ON decomposition to NH_4^+ in comparison to CHCF while effectively reducing NH_4^+ uptake via increased soil rhizosphere's alkalinization. A slow-release, granular NPK fertilizer limited soil rhizosphere's alkalinization via the inhibition of SOTCF. High TCF was the key factor for the true alleviation of NH_4^+ toxicity in NH_4^+ sensitive short-term crops even without the existence of a slow-release, granular mechanism, providing controlled uptake of available inorganic-N sources for optimal plant growth.

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