horticulturae

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Effects of Neem Seed Extract on Nitrate and Oxalate Contents in Amaranth Fertilized with Mineral Fertilizer and Cricket Frass

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Abstract: A vegetable's high antinutrients, nitrate (NO_3^-) and oxalate, could be remediated by neem seed extract. The combined use of neem seed extract with mineral fertilizer and cricket frass was conducted to evaluate their effects on amaranth's tissue NO_3^- and oxalic acid contents by inhibiting nitrification. The effects of five soil amendments were investigated: unamended, mineral fertilizer, and three rates of cricket frass (3.125 Mg ha^{-1}, 6.25 Mg ha^{-1}, and 12.5 Mg ha^{-1}), combined with two rates of neem seed extract: without (−Nm) and with (+Nm) extract. Only the neem extract applied to soils receiving mineral fertilizers decreased soil tissue NO_3^-−N contents (0.82 g kg^{-1} for −Nm vs. 0.62 g kg^{-1} for +Nm). The oxalic acid content of amaranth decreased with mineral fertilizer (0.60 and 0.46 g kg^{-1} for −Nm and +Nm, respectively), yet increased with the higher rates of cricket frass (1.42–1.52 g kg^{-1} for −Nm, and 1.23–1.51 g kg^{-1} for +Nm) compared to the unamended soil (1.05 and 1.00 g kg^{-1} for −Nm and +Nm). Cations, including K, Ca, Mg, and Na derived from cricket frass, may enhance biosynthesis and the accumulation of oxalic acid. The neem seed extract decreased the tissue's oxalic contents regardless of soil amendments.

Keywords: antinutrient; azadirachtin; natural nitrification inhibitor; nimbolide; nitrogen-rich organic amendment

Citation: Sriraj, P.; Toomsan, B.; Butnan, S. Effects of Neem Seed Extract on Nitrate and Oxalate Contents in Amaranth Fertilized with Mineral Fertilizer and Cricket Frass. *Horticulturae* **2022**, *8*, 898. https://doi.org/10.3390/horticulturae8100898

Academic Editors: Othmane Merah, Purushothaman Chirakkuzhyil Abhilash, Magdi T. Abdelhamid, Hailin Zhang and Bachar Zebib

Received: 25 August 2022
Accepted: 27 September 2022
Published: 29 September 2022

1. Introduction

While leafy vegetables are known to be essential for a healthy diet [1], amaranth is a favorite, consumed worldwide, as it contains a variety of nutrients [2]. Amaranth however also owns high contents of antinutrients, i.e., nitrate (NO_3^-) and oxalate [3], in particular that grown in soils amended with mineral fertilizer [4]. Nitrate is risky because it is a precursor of *N*-nitroso compounds, resulting in severe health risks, such as cancer, methemoglobinemia, hyperthyroidism, and diabetes [5]. Vegetables high in oxalates may bring about acute syndromes, such as mouth and tongue irritations, convulsions, and chronic syndromes, such as mitochondrial dysfunction, hyperoxaluria, hemorrhage, hematuria, hypocalcemia, renal failure, and renal kidney stones [6,7].

Mineral fertilizers have been reported to increase plant NO_3^- contents [4]. Increased tissue NO_3^- content drives cells to raise alkaline levels during nitrate reduction, which prompts plants to produce greater organic acids to balance cell pH [8]. The primary organic acid used to regulate the pH of amaranth cells is oxalic acid [9,10]. Tissue NO_3^- content was reported to decrease through the use of organic amendments due to lower N availability [4,11]; however, the results were unfavorable, as it created a nitrogen (N) deficiency due to insufficient N supply [12]. Higher qualities are therefore needed in the further utilization of organic amendments. Cricket frass, which is the cricket excrement, is a promising, high-quality organic amendment, due to its high N content, thereby prompting the emergence of cricket farms throughout Asia and Africa [13]. However, the rapid rate

of N release via the nitrification of the rich-N organic amendments, *viz* cricket frass, may create high concentrations of NO_3^- in soil and vegetables relative to the lower N-containing organic amendments [14], which can be addressed by slowing the nitrification rate. Several nitrification inhibitors are practically used, but they are expensive and unavailable in traditional markets [15]. Therefore, the use of an inexpensive and natural product, like neem seed extract, is not only cost-saving but also environmentally friendly. Azadirachtin and nimbolide, the most important active ingredients in neem (*Azadirachta indica*) extract, have been investigated as natural nitrification inhibitors, as they hinder the nitrifiers [16]. However, to our knowledge, remediation of vegetable NO_3^- and oxalate contents through natural nitrification inhibitors in soil amended with an N-rich organic amendment utilizing neem seed extract has yet to be reported.

The study herein addresses the hypotheses that cricket frass would decrease tissue NO_3^- and oxalate contents of amaranth, and that further pronounced decreases would occur through the amendment of neem seed extract. The objectives of the current study, therefore, were to evaluate the combinations of various soil amendments and neem seed extract on soil nitrification and amaranth's tissue NO_3^- and oxalate contents.

2. Materials and Methods

2.1. Soil, Cricket Frass, and Neem Seed Extract

The soil used in this study was collected at a depth of 0–15 cm from a research field in Sakon Nakhon Rajabhat University, Sakon Nakhon province, Thailand (17°11′09.7″ N; 104°05′18.8″ E). The soil was air-dried and sieved through a 2-mm mesh before use in the experiments herein. The initial soil properties are shown in Table 1.

Table 1. Initial characteristics of soil and cricket frass used in the experiment.

Characteristic	Soil	Cricket Frass
Soil particle distribution		
Sand (%)	78.70	-
Silt (%)	15.79	-
Clay (%)	5.51	-
Soil texture	Loamy sand	-
Bulk density (g cm^{-3})	1.51	0.33
pH (1:1)	5.62	8.21
Electrical conductivity (mS cm^{-1})	0.093	14.95
Organic C (g kg^{-1})	4.19	185.1
Total N (g kg^{-1})	0.38	43.5
NH_4^+–N (mg kg^{-1})	0.238	1436
NO_3^-–N (mg kg^{-1})	2.1	10.5
P (mg kg^{-1})	52.5	3039
K (mg kg^{-1})	34.0	18,720
Ca (mg kg^{-1})	301	1562
Mg (mg kg^{-1})	35	2052
Na (mg kg^{-1})	73	3920
Al (mg kg^{-1})	52.2	nd

nd = not detectable.

Cricket (*Acheta domesticus*) frass was obtained from a cricket farm in Sakon Nakhon, Thailand. The cricket frass was air-dried, cleaned, and sieved through a 2-mm mesh before use. The cricket frass characteristics are presented in Table 1.

Neem (*Azadirachta indica*) seed extract, a natural nitrification inhibitor, was produced from neem seed cake by a mill factory in Nakhon Ratchasima, Thailand, as a commercial product available in traditional markets, and contained 1.35 g N L^{-1}, 1.38% azadirachtin, and 0.034% nimbolide.

2.2. Greenhouse Experiment

A pot experiment was conducted from January to February 2020 under greenhouse conditions. Equipped with an evaporative cooling system, the mean air temperature of the greenhouse was 28.4 °C. A factorial arrangement (5 × 2) was conducted in a completely

randomized design with three replications. The experiment consisted of unamended (Un), mineral fertilizer (MF), and three rates of cricket frass: 3.125 Mg ha^{-1} (CrF_{Low}), 6.25 Mg ha^{-1} (CrF_{Medium}), and 12.5 Mg ha^{-1} (CrF_{High}). Two rates of neem seed extract, without (−Nm) and with (+Nm) extract, were combined with these soil amendments.

Amaranth (*Amaranthus tricolor*) was seeded and nursed in a plug tray for 15 days before a single seedling was transplanted into each pot. A pot (d = 15 cm, h = 14 cm, and V = 2085 cm^3) was filled with 2 kg of air-dried soil. Mineral fertilizer grades; 46–0–0, 18–46–0, and 0–0–60 at the rates of 0.02, 0.439, and 0.177 g pot^{-1}, respectively, were employed to achieve a recommended rate of 312.5 kg N ha^{-1}, 100 kg P ha^{-1}, and 100 kg K ha^{-1} [17], applied twice in equal portions into the accompanying pots on days 3 and 15 after the amaranth was transplanted. Cricket frass in the amounts of 2.76, 5.52, and 11.04 g pot^{-1}, equivalent to 3.125, 6.25, and 12.5 Mg ha^{-1}, respectively, were mixed thoroughly and incubated into their respective pots 15 days before the amaranth transplanting. The amounts of mineral fertilizer and cricket frass for each accompanying application rate were calculated based on the initial bulk density of the soil (1.51 g cm^{-3}). Each +Nm pot received 50 mL of neem seed extract, modified from that proposed in Sarawaneeyaruk, et al. [18], at a concentration of 0.1 mg azadirachtin mL^{-1} once a week at 15, 22, 29, and 36 days after planting. Soil water content was maintained with distilled water at 19.04% w/w (380.8 g pot^{-1}), equivalent to 65% of soil water holding capacity, by weighing each pot daily throughout the experiment. The aboveground biomass of amaranth was cut on day 39, under the presumption that it might have gained the maximum NO_3^- and oxalate contents [19,20]. Fresh soil sampling was undertaken for further determination of the NH_4^-, NO_3^-, and microbial numbers. The remaining soil was allowed to air dry and sieved through a 2-mm mesh for additional soil property analyses.

2.3. Laboratory Analyses

Soil particle size distribution and texture were achieved via the pipette method. Soil bulk density was examined according to the core method, and soil water holding capacity was determined following the method of maximum water-holding capacity.

Soil pH and electrical conductivity were assessed using the soil-to-water ratios of 1:1 and 1:5 w/v, respectively. Soil organic carbon determination was performed using the Walkley and Black method [21]. Soil total nitrogen was determined according to the micro-Kjeldahl method [22]. Soil NH_4^+ and NO_3^- were determined using fresh soil by extraction in 2 *M* KCl, and measured through the distillation method [22] on a micro-Kjeldahl distillator (Pro-Nitro S 4002851, JP Selecta, Barcelona, Spain). Soil phosphorus was extracted in Bray-2 solution and revealed via a UV-Vis spectrophotometer (Hitachi U-5100, Hitachi High-Tech Corporation, Tokyo, Japan) using a wavelength of 820 nm [22]. Soil K, Ca, Mg, and Na were extracted in 1 *N* NH4OAc at pH 7 [23] and measured on a flame atomic absorption spectrometer (Agilent 240FS AA, Agilent Technologies, Santa Clara, CA, USA). Exchangeable Al was extracted in 1 *M* KCl and determined by the titrimetry method following Pansu and Gautheyrou [23].

Azadirachtin and nimbolide concentrations of the neem seed extract and soil were determined using the high-performance liquid chromatography (HPLC) technique by modifying a method described by Stark and Walter [24]. Soil microbial numbers were determined using the plate count technique modifying a method described by Olsen and Bakken [25].

Amaranth's tissue NO_3^- content was determined following the salicylic acid assay of Cataldo, et al. [26], and oxalic acid was assessed using the HPLC technique following Rahman, et al. [27].

2.4. Data Calculation and Statistical Analyses

Net nitrification rates (Equation (1)) were calculated by modifying the formula of Bi, et al. [28]:

$$\text{Net nitrification rate}\left(\text{mg N kg}^{-1}\text{soil day}^{-1}\right) = \frac{[NO_3^- - N]_{t2} - [NO_3^- - N]_{t1}}{t} \quad (1)$$

where $[NO_3^- - N]_{t2}$ and $[NO_3^- - N]_{t1}$ are soil $NO_3^- - N$ concentrations (mg kg^{-1}) at the harvest and the start of the experiment, respectively, and t is the number of days from start to harvest.

Nitrification inhibition (Equation (2)) was calculated using an equation modified from Aspelin and Ekholm [29]:

$$\text{Nitrification inhibition } (\%) = \frac{(\text{Net nitrification rate})_{un} - (\text{Net nitrification rate})_{am}}{(\text{Net nitrification rate})_{un}} \quad (2)$$

where $(\text{Net nitrification rate})_{un}$ is the net nitrification rate of the Un treatments, and $(\text{Net nitrification rate})_{am}$ in the treatment of MF, CrF_{Low}, CrF_{Medium}, or CrF_{High}.

A two-way analysis of variance based on a factorial arrangement in a completely randomized design was used to evaluate the effects of soil amendments and neem seed extract on tissue NO_3^- and oxalic acid contents of amaranth and its related soil properties. Multiple comparisons were assessed through Tukey's honest significant difference test. Significant differences were at $p \leq 0.05$.

3. Results and Discussion

The neem seed extract was found to have an inhibitory effect on nitrification only in soil free of cricket frass, namely, that of the unamended and mineral fertilizer (Table 2). This effect, in turn, decreased the amaranth's tissue $NO_3^- - N$ concentrations in soil amended with mineral fertilizer (Table 3). The positive nitrification inhibition values of Un + Nm and MF + Nm, as well as the significantly lower $NO_3^- - N$ concentrations and net nitrification rates in Un + Nm and MF + Nm than Un - Nm and MF - Nm, offered evidence in favor of that assertion (Table 2). Active ingredients in the neem extract, particularly azadirachtin and nimbolide, played a crucial role in inhibiting ammonia-oxidizing microorganisms [16]. The existence of azadirachtin and nimbolide in only the unamended and minerally fertilized soil (Table 4) confirmed their functions in nitrification inhibition. Ammonia-oxidizing bacteria, such as *Nitrosospira*, *Nitrosomonas*, and *Nitrosococcus*; and ammonia-oxidizing archaea, like *Nitrosopumilus* and *Nitrososphaera* were identified as nitrifiers responsible for transforming NH_4^+ to NO_3^- in soil [30]. Nitrification inhibitors hindered ammonia-oxidizing microorganisms by inhibiting ammonia monooxygenase, which catalyzed the transformation reaction of NH_3 to NH_2OH [31].

Table 2. Soil mineral nitrogen (NH_4^+-N and NO_3^--N), net nitrification rate, and nitrification inhibition as affected by the combined uses of different rates of cricket frass and neem seed extract.

Amendment †	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Net Nitrification Rate (mg N kg^{-1}Soil Day^{-1})	Nitrification Inhibition (%)
Un − Nm	2.04 c ‡	2.08 ab	0.034 ab	–
Un + Nm	0.74 d	1.21 d	0.012 d	65.6 b
MF − Nm	2.00 c	1.56 c	0.021 c	–
MF + Nm	0.74 d	0.80 e	0.001 e	93.7 a
CrF_{Low} − Nm	1.71 c	0.99 de	0.006 de	–
CrF_{Low} + Nm	1.87 c	1.53 c	0.020 c	−227.8 d
CrF_{Medium} − Nm	1.9 c	1.56 c	0.021 c	–
CrF_{Medium} + Nm	2.67 b	2.00 b	0.032 b	−54.1 c
CrF_{High} − Nm	2.55 b	1.91 b	0.030 b	–
CrF_{High} + Nm	3.57 a	2.29 a	0.040 a	−44.5 c
p-value	<0.001	<0.001	<0.001	<0.001
F-test	***	***	***	***
CV (%)	7.08	4.97	9.39	−14.78

*** = $p \leq 0.001$. † Un = unamended; MF = mineral fertilizer; CrF_{Low}, CrF_{Medium}, and CrF_{High} = cricket frass at rates of 3.125, 6.25, and 12.5 Mg ha^{-1}, respectively. ‡ Means within the same column followed by the same letter are not significantly different at $p \leq 0.05$ (Tukey's honest significant difference test).

Table 3. Shoot fresh biomass, tissue NO_3^-−N, and oxalic acid contents of amaranth as affected by the combined uses of different rates of cricket frass and neem seed extract.

Amendment †	Shoot Fresh Biomass	Tissue NO_3^-−N		Oxalic Acid
	(g Plant^{-1})	Content (g kg^{-1})	Change (%) §	(g kg^{-1})
Un − Nm	6.3 d ‡	0.69 b	0.0	1.05 d
Un + Nm	4.6 d	0.59 b–d	−14.5	1.00 d
MF − Nm	18.7 c	0.84 a	+21.7	0.60 e
MF + Nm	14.8 c	0.62 bc	−10.1	0.46 f
CrF_{Low} − Nm	26.1 b	0.55 cd	−20.3	1.42 ab
CrF_{Low} + Nm	26.3 b	0.54 cd	−21.7	1.23 c
CrF_{Medium} − Nm	39.9 a	0.61 b–d	−11.6	1.45 ab
CrF_{Medium} + Nm	38.7 a	0.69 b	0.0	1.38 b
CrF_{High} − Nm	28.7 b	0.48 d	−30.4	1.52 a
CrF_{High} + Nm	19.3 c	0.54 cd	−21.7	1.51 a
p-value	<0.001	<0.001		<0.001
F-test	***	***		***
CV (%)	8.65	7.44		5.38

*** = $p \leq 0.001$. † Un = unamended; MF = mineral fertilizer; CrF_{Low}, CrF_{Medium}, and CrF_{High} = cricket frass at rates of 3.125, 6.25, and 12.5 Mg ha^{-1}, respectively. ‡ Means within the same column followed by the same letter are not significantly different at $p \leq 0.05$ (Tukey's honest significant difference test). § Changes (%) in tissue NO_3^-−N content are relative to the Un−Nm.

Table 4. Azadirachtin and nimbolide concentrations in soil at the completion of the experiment as affected by the combined uses of different rates of cricket frass and neem seed extract.

Amendment †	Azadirachtin Concentration (mg kg^{-1})	Nimbolide Concentration (mg kg^{-1})
Un − Nm	–	–
Un + Nm	9.36 b ‡	0.25 b
MF − Nm	–	–
MF + Nm	15.33 a	0.48 a
CrF_{Low} − Nm	–	–
CrF_{Low} + Nm	0 c	0 c
CrF_{Medium} − Nm	–	–
CrF_{Medium} + Nm	0 c	0 c
CrF_{High} − Nm	–	–
CrF_{High} + Nm	0 c	0 c
p-value	<0.001	<0.001
F-test	***	***
CV (%)	13.68	17.08

*** = $p \leq 0.001$. † Un = unamended; MF = mineral fertilizer; CrF_{Low}, CrF_{Medium}, and CrF_{High} = cricket frass at rates of 3.125, 6.25, and 12.5 Mg ha^{-1}, respectively. ‡ Means within the same column followed by the same letter are not significantly different at $p \leq 0.05$ (Tukey's honest significant difference test).

The inhibitory effects of neem seed extract were not shown in soil amended with cricket frass, due to the disappearance of the extract's active ingredients, azadirachtin and nimbolide (Table 3). Azadirachtin and nimbolide vanished due to hydrolytic [32] and microbial degradations [24,33]. In this study, increased soil pH (Table 5) and the increased number of soil microorganisms with increasing cricket frass rates (Table 6) confirmed the denature of azadirachtin and nimbolide through hydrolytic and microbial degradation, respectively. Sundaram, et al. [32] asserted that in hydrolytic degradation, azadirachtin's carboxylic ester groups and epoxide rings underwent acyl-O cleavage via a base-catalyzed reaction, due to increased pH, converting the ester group (RCO_2R') to a carboxylate group ($RCOO^-$). Even though pH in soils receiving cricket frass was not alkaline, denatures of azadirachtin and nimbolide could be a result of the hydrolytic degradation manifested by increased soil pH. In terms of microbial degradation, the increased number of soil microorganisms in this study was consistent with the findings of Agyarko, et al. [33], which revealed that the rate of azadirachtin degradation was faster in soil amended with higher amounts of cow and poultry manures. It was a possibility that the inhibitory property might have functioned through receiving the

additional neem seed extract after the microbial population declined. However, this means no more cricket frass had been amended to the soil.

Table 5. Soil chemical properties as affected by the combined uses of different rates of cricket frass and neem seed extract.

Amendment †	pH (1:1)	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})
Un − Nm	5.89 b ‡	53.3 e	32.0 b	255 de	24.6 cd	35.0 a
Un + Nm	6.05 b	56.7 de	32.0 b	308 cd	22.9 cd	34.0 ab
MF − Nm	5.54 c	80.4 c	36.0 b	250 e	19.7 d	34.0 ab
MF + Nm	5.64 c	76.7 c	34.0 b	276 de	20.4 d	33.0 bc
CrF_{Low} − Nm	5.98 b	78.3 c	34.0 b	351 bc	27.5 c	32.0 c
CrF_{Low} + Nm	5.92 b	75.0 cd	33.0 b	277 de	27.5 c	30.0 d
CrF_{Medium} − Nm	5.93 b	92.5 c	36.0 b	297 c–e	32.9 b	30.0 d
CrF_{Medium} + Nm	5.99 b	91.7 c	37.0 b	292 de	35.6 b	30.0 d
CrF_{High} − Nm	6.54 a	340.6 a	94.5 a	436 a	73.5 a	28.0 e
CrF_{High} + Nm	6.48 a	318.8 b	90.0 a	372 b	69.1 a	28.0 e
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
F-test	***	***	***	***	***	***
CV (%)	1.44	5.09	3.79	6.17	4.95	1.42

*** = $p \leq 0.001$. † Un = unamended; MF = mineral fertilizer; CrF_{Low}, CrF_{Medium}, and CrF_{High} = cricket frass at rates of 3.125, 6.25, and 12.5 Mg ha^{-1}, respectively. ‡ Means within the same column followed by the same letter are not significantly different at $p \leq 0.05$ (Tukey's honest significant difference test).

Table 6. Soil microbial number indicated by the colony-forming unit (CFU) as affected by different rates of cricket frass and neem seed extract.

Amendment †	×10^{9} CFU kg^{-1} soil
Un − Nm	3.57 d ‡
Un + Nm	2.73 e
MF − Nm	4.38 b–d
MF + Nm	3.79 cd
CrF_{Low} − Nm	4.50 bc
CrF_{Low} + Nm	4.53 bc
CrF_{Medium} − Nm	4.98 b
CrF_{Medium} + Nm	4.84b
CrF_{High} − Nm	5.93 a
CrF_{High} + Nm	5.93 a
p-value	<0.001
F-test	***
CV (%)	6.37

*** = $p \leq 0.001$. † Un = unamended; MF = mineral fertilizer; CrF_{Low}, CrF_{Medium}, and CrF_{High} = cricket frass at rates of 3.125, 6.25, and 12.5 Mg ha^{-1}, respectively. ‡ Means within the same column followed by the same letter are not significantly different at $p \leq 0.05$ (Tukey's honest significant difference test).

Despite the dysfunctional results of the neem extract, cricket frass alone decreased tissue NO_3^--N concentrations by 11.6–30.4%, but that of the mineral fertilizer alone increased by 21.7% (Table 3). The advantage of organic fertilizer, *viz* the cricket frass herein, in remediation of NO_3^-−N content in the plant, was consistent with the findings of Zandvakili, et al. [4]; which demonstrated that the application of cow manure (100–800 mg N kg^{-1}) produced 34–88 mg NO_3^-−N kg^{-1} dry weight of lettuce, which decreased NO_3^-−N by 78–99%, compared to the urea at the same nitrogen rates, producing 153–6085 mg NO_3^-−N kg^{-1} dry weight. The current study employed urea and ammonia fertilizer as its N-source. These fertilizers hydrolyzed readily to NH_4^+ and nitrified rapidly to NO_3^-, making them readily available for plant uptake. However, the N derived from the organic cricket frass utilized herein was slowly ammonified and nitrified to NH_4^+ and NO_3^-, respectively [34].

High NH_4^+−N content of cricket frass (Table 1), which should have been nitrified to NO_3^--N before plant uptake, did not translate into high tissue NO_3^-−N content (Table 3). The results might be due to that fact that NH_4^+ was lost as NH_3 volatilization due to higher pH of soil amended with the frass (Table 5), which was the alkaline nature of cricket frass

(Table 1), compared to mineral fertilizer. Results of this study were in line with those of Swelum, et al. [35], who claimed that NH_3 volatilization from poultry manure, a rich-N organic material, was immediately high since the first day of the application, and the losing rate was depended crucially upon soil pH.

Mineral fertilizer decreased the oxalic acid contents in amaranth, which was more pronounced when neem seed extract was applied (Table 3). The current study applied NH_4^+-producing compounds derived from urea and ammoniacal fertilizers. The NH_4^+-producing compounds were demonstrated to decrease plant oxalic acid content relative to nitrate fertilizer [36,37]. Moreover, nitrification inhibition was more prominent when combined with the use of neem seed extract with mineral fertilizers, which manifested in lower soil NO_3^-−N concentrations in MF+Nm than MF−Nm (Table 2). Rahman and Kawamura [8] put forward that OH^- and H^+ produced during plant N assimilations affected oxalate contents. A large quantity of OH^- is produced in plants' nitrate and nitrite assimilations when soil obtains a large portion of NO_3^-. Increases in OH^- in plant cells stimulated the biosynthesis of organic anions, especially the oxalic acid in amaranth, and brought about the dissociation of H^+ from those organic acids creating negative charges upon their R-COO^- [9].

Cricket frass significantly increased the amaranth's oxalic acid contents in amounts incremental with the increased frass rates (Table 3). Cations, i.e., K, Ca, Mg, and Na, were found to stimulate the biosynthesis of oxalic acid in plants, which corresponded to the generally accepted cation-anion balance [38]. As per this study's findings, cricket frass was an important source of these cations (Table 1), and, as the rate of the frass increased, so increased the oxalic acid contents in the amaranth (Table 3). Additionally, under the treatment of neem seed extract, oxalic acid content tended to decrease with low and medium rates of cricket frass (Table 3). According to Schubert and Yan [39], NH_4^+ induced plant H^+ production, which in turn caused the cytoplasm to become more acidic. Under the cricket frass amendments within this study, NH_4^+ was also gained from degraded neem extract, producing increased soil NH_4^+−N concentrations under the neem extract treatments (Table 2). This could prohibit plants from synthesizing organic anions, including oxalic acid [8].

4. Conclusions

The results of this study demonstrate that neem seed extract exhibited inhibitory properties in only the unamended and minerally fertilized soils, but not in soil amended with cricket frass. Denatures of the neem's active ingredients (azadirachtin and nimbolide) were responsible for the inactive properties of the neem extract. Despite the extract's lack of inhibitory activity, utilizing cricket frass decreased amaranth's NO_3^-−N contents by 11.6–30.4%. In contrast, mineral fertilizer produced a 21.7% increase in tissue NO_3^-−N.

The oxalic acid content of amaranth decreased with mineral fertilizer utilization, whereas the cricket frass amendments produced the opposite effect. Cricket frass-derived cations may have stimulated the biosynthesis and accumulation of oxalic acid. Rising application rates of cricket frass increased the amaranth's oxalic acid content. Tissue oxalic acid contents of amaranth were typically decreased using neem seed extract throughout soil amendments of both mineral fertilizer and cricket frass, as this extract increased soil NH_4^+ concentrations, which may have hindered the amaranth in synthesizing oxalic acid. Further research is required on the temporal changes of the active ingredients of neem seed extract in soil with mineral and organic fertilizers.

Author Contributions: S.B. designed and conducted the research and prepared and drafted the manuscript. P.S. conducted research, proofread, and improved the manuscript. B.T. designed the research, proofread, and improved the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Research Fund for Researchers from Revenue FY 2020 (Project no. 7/2563) and the Research Career Development Fund FY 2022 (Project no. 7/2565) from Sakon Nakhon Rajabhat University.

Data Availability Statement: Not applicable.

Acknowledgments: The authors wish to thank Janista Duangpukdee and Dareena Jaiseree for coordinating the data collection.

Conflicts of Interest: The authors declare no conflict of interest.

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