



## Research Article

### Effects of Cricket Frass Biofertilizer on Growth of Spring Onion (*Allium Fistulosum L.*) and Physicochemical Properties of Soil

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Received: May 20, 2022 Revised: July 7, 2022 Accepted: July 26, 2022 Published: September 15, 2022

#### Abstract

**Objective:** This study aims to assess the effects of cricket frass biofertilizer on the growth and yield potential of spring onion (*Allium fistulosum L.*) and its residual effects on physicochemical properties of the soil in greenhouse and field conditions.

**Methods:** 7 treatments namely cricket frass (0t/Ha, 5t/Ha, 10t/Ha, 15t/Ha, and 20t/Ha), poultry manure (15t/Ha) and cattle manure (15t/Ha) were used and replicated 3 and 4 times in field and greenhouse conditions respectively. Plots of 1m<sup>2</sup> arranged in a randomized complete block design for field experiment and pots with 5kg of soil laid out in a completely randomized design for the controlled experiment were used. Spring onion transplants were maintained in both trials for 16 weeks with uniform agronomic inputs. Growth and yield were assessed based on plant height, crop growth rate, number of leaves per plant, plant fresh weight, plant dry weight and root to shoot ratio. The effect of frass on soil was scored based on the percentage of organic carbon, nitrogen, phosphorus, and potassium at the start and end of the experiment. The data was assessed for normality (Shapiro-Wilks test) and subjected to analysis of variance at  $P < 0.05$  using R-statistical software. Treatment means were separated using fishers-protected least significant different.

**Results:** Frass application increased growth and yield traits in both trials with the exception of the number of leaves per plant, regardless of the dosage. Frass at 15t/ha outperformed all other treatments and competed favorably against conventional manures obtained from poultry and cattle. Frass bio-fertilizer significantly increased the organic carbon, phosphorus, potassium, calcium and magnesium content of the soils. Frass contained adequate nutrients in labile forms that contributed to growth and yields. This significance can also be linked to the presence of growth promoters and stimulation of microbial population in soils, which is characteristic of insect manures. The high levels of nutrients in the soil after planting confirms frass as an amicable soil amendment that buffers and supports soil properties.

**Conclusion:** Cricket frass biofertilizer is a valuable bio-fertilizer that has the potential to improve the quality of soils and support the growth and yields of notable kitchen vegetables such as onions.

**Keywords:** bio-fertilizer, cricket frass, growth, onion, soil, yields

**Citation:** Ogaji SO, Watako AO, Nyongesah JM, Peter B. Effects of Cricket Frass Biofertilizer on Growth of Spring Onion (*Allium Fistulosum* L.) and Physicochemical Properties of Soil. *J Mod Agric Biotechnol*, 2022; 1(3): 14. DOI: 10.53964/jmab.2022014.

## 1 INTRODUCTION

Agricultural soils in many regions of the world, especially in developing regions, continue to lose fertility at alarming rates. Soils in Sub-Saharan Africa, for instance, have lost 660, 75, and 450kg ha<sup>-1</sup> of nitrogen (N), phosphorus (P) and potassium (K) respectively within the last 3 decades alone<sup>[1]</sup>. The consequence of this inefficient management of soils is costing the region more than US \$4 billion in nutrient mining and US \$42 billion in losses per year. This has become the largest contributor to diminishing per capita food production<sup>[2]</sup>. Smallholder farmers, responsible for over 80% of food production in the region<sup>[3]</sup>, bear the brunt of this constraint due to lack of intervention strategies to address the challenges associated with the poor and degraded soils<sup>[3]</sup>. The rising costs of inorganic fertilizers make their application unsustainable in regions with effective research and extensive services due to limited financial resources of smallholder farmers in the region. Although chemical fertilizers positively impact crop productivity, their heavy and continuous use in commercial agriculture is responsible for increased soil acidity, reduced soil organic carbon (OC) composition and cation exchange capacity (CEC), and nutrient imbalances secondary to fixation and suppression of soil microbial activity in many parts of the world<sup>[4-7]</sup>. Additionally, environmental degradation in the form of salinization of soils, eutrophication of water, accumulation of heavy metals, water and air pollution, and disruption of ecosystems are all linked to over-reliance on inorganic fertilizers. Inorganic fertilizers have significant negative environmental impacts due to emissions from fossil fuels from which they are produced.

Food security in poorer regions of the world depends on improved productivity of smallholder farmers since they account for over 80% of the food produced. To realize the full potential of smallholder farmers while protecting our environment, it is therefore imperative to advocate and promote the use of sustainable, low-cost and efficient nutrient management systems that are tailored to their socio-economic status. Organic fertilizers are not only affordable, but are also alternative eco-friendly inputs for improving soil and crop productivity without any negative consequences to the environment and the surroundings<sup>[3]</sup>. Organic manures derived from withered plant parts, animal waste, and livestock by-products have been found to improve soil properties and crop yields while at the

same time protecting the soil, water and climate<sup>[8]</sup>. They are excellent sources of N, which is one of the important limiting soil nutrients for crops in different soil types<sup>[9]</sup>. Furthermore, use of organic manures is associated with an increase in soil organic matter, pH buffering, improvement of water holding capacity, nutrient cycling and CEC, and enhanced soil biological activities<sup>[2,10]</sup>. The gelling effect of organic manure on soil particles also promotes nutrient availability, uptake and utilization. Recently, several reports on the potential of insect waste as sources of biofertilizer have been published. Soil C and N dynamics were influenced by frass following gypsy insect defoliation in forest ecosystems<sup>[11,12]</sup>. High nutrient content, dense microbiota and plant growth promoters with the potential for use as biofertilizers have been reported in mealworm frass, which comprises a mixture of droppings (feces), shed exoskeletons and waste feed<sup>[13-16]</sup>. A number of studies have also reported significant increases in growth and yields of several crops such as maize<sup>[17,18]</sup>, spring onions<sup>[19]</sup>, chili, pepper and shallots treated with black soldier fly frass as a bio-fertilizer<sup>[20]</sup>. However, some studies have also reported some negative effects of frass on crop performance. Stunting was reported on maize crops in soils amended with untreated black soldier fly frass<sup>[21]</sup>. Allelopathy was recorded on *Brassica rapa* and lettuce treated with frass from *Mamestra brassicae*<sup>[22]</sup> and cerambycid larvae (*Chlorophorus annularis*)<sup>[23]</sup> respectively. Ammonium and N toxicities have also been associated with the use of frass<sup>[21]</sup>.

The increasing demand for insects as alternative livestock<sup>[24,25]</sup> coupled with their ease of domestication and prospect for mass production has led to emergence of small and medium-scale insect industries that produce relatively large quantities of frass as a byproduct<sup>[13,15]</sup>. Insect frass is often unused or just disposed as waste. Although insects such as crickets are efficient converters of feed resources, the amount of waste they produce (~33% of feed consumed) is still significant<sup>[22,24,26]</sup> and cannot be disregarded as negligible, particularly in an era where feed resource is constraining and the predominant cricket substrate remains commercial chicken feed which is in itself inadequate and prohibitive<sup>[24,27]</sup>. The high value of nutrients with high N concentrations in frass<sup>[28]</sup> are convertible to inorganic forms such as ammonia and nitrates<sup>[11]</sup>, thus giving frass the potential to be used as a biofertilizer in agriculture. Frass from house cricket (*Acheta domesticus*) contains 2.27%,

2.02% and 2.26% total N, P and K respectively, with traces of micronutrients including calcium (Ca), sodium (Na) and magnesium (Mg). This compares favorably with conventional animal manures obtained from animals such as cattle, poultry and pigs<sup>[13,24]</sup>. The value of frass as a source of nutrients for crops is not new, as evidenced by past encouragement of farmers to spread frass in their fields after deactivation<sup>[15]</sup>. However, there is still limited literature on the effects of frass on growth and performance of many crop species, as well as its impact on the physicochemical properties of the soil.

Onion is an important vegetable crop that requires high amounts of N for it to grow<sup>[29,30]</sup>, regardless of the source. Thus, onion production systems that rely on inorganic fertilizers significantly contribute to environmental degradation and pollution. Use of insect frass as an alternative source of nutrients for onion would not only present an innovative strategy that addresses the problem of insect frass contamination and safety of the environment as well as farmers, but would also align with circular economic principles whereby waste from insect farming is processed and re-used to boost agricultural productivity<sup>[15]</sup>. This study therefore evaluated the residual effects of frass derived from house and field crickets on growth and yield traits of spring onions (*Allium fistulosum* L.), and its impact on the physicochemical properties of the soil under field and greenhouse condition.

## 2 MATERIALS AND METHODS

### 2.1 Study Site

The experiment was conducted within the months of March to August 2020, at the experimental farm of Jaramogi Oginga Odinga University of Science and Technology (JOOUST) in Bondo, Kenya, which is located at latitude -0.093889 and longitude 34.258611. The climate is strongly influenced by the expansive Lake Victoria which influences the distribution and amount of rainfall. The mean annual rainfall ranges from 800 to 1600mm, and occurs in a bi-modal distribution with long rains between March and August, and short rains between September and November. The average temperature is 22.5°C. Bondo area is characterized by different soils, with clayish acrisols representing the predominant soil type<sup>[31]</sup>.

### 2.2 Manure Preparation

Cricket frass comprising of droppings from both house cricket (*Acheta domesticus*) and field cricket (*Gryllus bimaculatus*) was obtained from JOOUST insect farm. The crickets were predominantly fed on chicken grower/layer mash supplemented with leaves of kale (*Brassica oleracea*) and/or sweet potatoes (*Ipomoea batatas*). Cattle manure (CM) and Poultry manure (PM) were collected from JOOUST dairy farm and Kopoda farm within Bondo, respectively. The PM used was from a deep litter system representing a mix of bedding materials that

included saw dust. All manures were further decomposed (hot composting) for 3 months to ensure complete decomposition, sorted and sieved through a 2mm mesh. Random samples of manure were separately collected from the bulks for laboratory analysis. The composition of manures used in the trial is shown in Table 1.

### 2.3 Methodology

Treatments of 5t/ha, 10t/ha, 15t/ha, and 20t/ha of frass (FR5, FR10, FR15, and FR20), 15t/ha of PM (PM15) and 15t/ha of cattle manure (CM15), and control of zero organic fertilizer application (FR0) were used in a randomized complete block design for field experiments with 3 replications, and completely randomized design for greenhouse experiments with 4 replications. Field plot sizes were 1m×1m raised beds separated by a spacing of 50cm, while the greenhouse experiments consisted of uniform pots filled with 5kg of 2mm mesh-sieved soil on metallic benches. Treatments were incorporated into soils uniformly 2 weeks before transplanting. 28 days old seedlings of “green bunching” spring onion hybrid variety from Amiran Kenya were transplanted in pairs per pot for the greenhouse experiment, and 36 per plot with 20cm round spacing for the field experiment. Agronomic practices, including watering at 60% field capacity every 2 days, were uniform for all treatments in both the greenhouse and field experiments.

### 2.4 Data Collection

#### 2.4.1 Plant Measurements

Data was collected from all plants per pot in the greenhouse house experiment. In the field experiment, data was collected from the same randomly selected and tagged 4 plants per plot to ensure consistency of the data. Plant height was measured as distance from the base of soil to the apex of tallest leaf every 7 days after transplanting (DAT). Crop growth rate (CGR) was calculated using the formula  $[(H_x - H_y)/t_{x-y}]$  where  $H_x$ =height of the plant  $x$  days after transplanting;  $H_y$ =height of the plant  $y$  days after transplanting;  $t_{x-y}$ =number of days between  $x$  and  $y$ . Number of leaves were counted at 28, 49 and 63 DAT. At 70d after transplanting (70 DAT), all plants were harvested and weighed for fresh weight (FWT) before oven drying for 48h at 60°C followed by dry weight (DWT) measurement. The shoots and roots of oven-dried plants were separated and weighed to determine the root to shoot ratio (RSR).

#### 2.4.2 Soil Physicochemical Properties

4 soil samples were randomly collected from 4 middle rows of each field plot at a depth of 0-20cm using auger, while a representative sample was collected from each pot in the greenhouse 70 DAT. Composite soils were obtained from homogenized collected samples, and analyzed using previously described methods<sup>[32]</sup>. Soil OC was analyzed using sulfuric acid and aqueous K dichromate mixture method. The available N was analyzed using the

**Table 1. Properties of Pre-treated Soil and Manures Used in the Experiment**

Variable	EC (mS/M)	pH (W)	OC (%)	Total N (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)
Soil	7.65	8.6	1.26	0.3155	374	536	11329	339
FR	247	8.4	3.98	0.3482	1603	9461	42576	6967
PM	87.1	8.3	3.38	0.0054	1109	7346	133698	8217
CM	98.4	6.2	3.52	0.0177	557	2922	86476	191

Notes: FR: Cricket frass; PM: Poultry manure; CM: Cattle manure; EC: Electrical conductivity; OC: Organic carbon content; N: Nitrogen; P: Phosphorus; K: Potassium; Ca: Calcium; Mg: Magnesium.

Kjeldahl method, whereas the available P and K were analyzed using the ammonium lactate acid extract-based spectrophotometry and flame emission spectrophotometry methods. We measured pH of untreated soils and treated soils before and after the experiment. Electrical conductivity was determined based on extracts of 1:10 and 1:2.5 (*w/v*) for organic manure-distilled water and soil-distilled water respectively. The data was subjected to analysis of variance using the R statistical package ver. 4.0.2 and means separated using Least significant different test at  $P < 0.05$  with the package Agricola.

### 3 RESULTS

#### 3.1 Characteristics of Experimental Soil and Manures

The characteristics of the soil at the experimental site and the organic manures used in this study are shown in [Table 1](#). The soil was comprised of 53.09, 20.42 and 25.40% sand, silt and clay respectively, and thus is classified as sandy clay loam based on the hygrometer readings. The pH was moderately alkaline with very low OC and N concentrations. The average concentration of K was 536ppm, and that of P was 374ppm.

#### 3.2 Effect of Frass on Growth Parameters

##### 3.2.1 Effect of Frass on Plant Height

The effects of the treatments on plant height are shown in [Table 2](#). No significant differences in plant height were observed among all treatments ( $P < 0.05$ ) in the first 21 and 28 DAT for greenhouse and field experiments respectively. Plants displayed significant differences in height 35 DAT to 70 DAT in the field experiment, with taller plants observed in FR15 and CM15. Plants in FR15 were consistently taller than plants in the control 42 DAT to 70 DAT. Similar results were observed in the greenhouse experiment where plants in FR15 and FR20 also consistently displayed taller heights compared to plants in the control 42 DAT to 70 DAT. Plants in all the manure treatments, including FR5, FR10, FR15, FR20, PM15 and CM15, were significantly taller than plants in the control from 42 DAT to 70 DAT.

##### 3.2.2 Effect of Frass on CGR

Manure treatments significantly influenced CGR in both field and greenhouse experiments ([Table 3](#)). No significant differences in CGR were observed among treatments within the first 7 DAT to 21 DAT in the field experiment.

In the greenhouse experiment, there were significant differences in CGR among the treatments throughout the experimental period. Field plots treated with 15t/ha of frass consistently displayed higher CGRs compared to those treated with other treatments. Similarly, plants in FR15 in the greenhouse experiment had significantly higher CGRs between 35 DAT and 49 DAT with an increase of 11.11% over the control. Treatments FR5, FR10 and FR20 also exhibited relatively higher CGRs in comparison to the conventional manure treatments of PM15 and CM15 at 14 DAT, 28 DAT, and 56 DAT intervals. A general trend of significant increases in the CGR of plants was observed in FR15, FR20, PM15, and CM15 relative to FR0 treatment, with no significant differences among these treatments.

#### 3.3 Effect of Frass on Yield Parameters

##### 3.3.1 Effect of Treatments on Number of Leaves per Plant (NLF)

Manure treatments displayed higher NLF compared to the control treatment in both experiments ([Table 4](#)). In the field experiment, statistical differences were only observed 28 DAT, with no differences through the remaining period up to 70 DAT. Plants in FR15, FR20 and PM15 had significantly higher NLF than the control. No significant differences in NLF were observed between FR15, FR20 and PM15 from 28 to 70 DAT. In the greenhouse experiment, FR15 and FR20 displayed significantly higher NLF than the control from 49 DAT to 70 DAT. Similar to the field experiment, no significant differences in NLF were observed between FR15, FR20 and PM15.

##### 3.3.2 Effects of Treatment on FWT

All treatments significantly influenced plant FWT at harvest in both the field and greenhouse experiments, with plants in FR15 exhibiting the highest FWT ([Table 5](#)). The FWT of plants in the control was not significantly different from that of plants in the other treatments in the field experiment. However, FWT in the control was significantly lower than that of the other treatments in the greenhouse experiment.

##### 3.3.3 Effects of Treatments on DWT

Significant differences in biomass accumulation among treatments were observed in both field and greenhouse experiments ([Table 5](#)). FR15 consistently displayed higher

**Table 2. Effects of Treatments on Plant Height (cm)**

Experiment	Trt	Days After Transplanting (DAT)									
		7	14	21	28	35	42	49	56	63	70
Field	FR0	21.12 <sup>a</sup>	21.48 <sup>a</sup>	22.98 <sup>a</sup>	23.36 <sup>a</sup>	23.77 <sup>bc</sup>	25.58 <sup>bc</sup>	29.98 <sup>bc</sup>	37.91 <sup>b</sup>	41.97 <sup>b</sup>	47.40 <sup>b</sup>
	FR5	21.61 <sup>a</sup>	21.91 <sup>a</sup>	23.03 <sup>a</sup>	23.14 <sup>a</sup>	24.03 <sup>bc</sup>	27.88 <sup>abc</sup>	34.88 <sup>abc</sup>	43.15 <sup>ab</sup>	47.83 <sup>ab</sup>	52.87 <sup>ab</sup>
	FR10	21.22 <sup>a</sup>	21.42 <sup>a</sup>	22.83 <sup>a</sup>	23.55 <sup>a</sup>	24.81 <sup>abc</sup>	29.10 <sup>ab</sup>	35.85 <sup>ab</sup>	41.84 <sup>ab</sup>	46.52 <sup>ab</sup>	51.01 <sup>ab</sup>
	FR15	21.55 <sup>a</sup>	21.77 <sup>a</sup>	22.81 <sup>a</sup>	23.74 <sup>a</sup>	26.58 <sup>ab</sup>	32.66 <sup>a</sup>	40.07 <sup>a</sup>	47.27 <sup>a</sup>	52.48 <sup>a</sup>	55.89 <sup>a</sup>
	FR20	20.66 <sup>a</sup>	20.68 <sup>a</sup>	21.77 <sup>a</sup>	23.19 <sup>a</sup>	24.60 <sup>bc</sup>	29.16 <sup>ab</sup>	33.93 <sup>abc</sup>	42.18 <sup>ab</sup>	47.75 <sup>ab</sup>	53.41 <sup>ab</sup>
	PM15	20.71 <sup>a</sup>	21.05 <sup>a</sup>	21.63 <sup>a</sup>	21.75 <sup>a</sup>	22.44 <sup>c</sup>	24.05 <sup>c</sup>	29.15 <sup>c</sup>	37.43 <sup>b</sup>	41.83 <sup>b</sup>	47.77 <sup>ab</sup>
	CM15	22.29 <sup>a</sup>	23.43 <sup>a</sup>	24.68 <sup>a</sup>	25.44 <sup>a</sup>	27.96 <sup>a</sup>	31.21 <sup>a</sup>	34.46 <sup>abc</sup>	41.47 <sup>ab</sup>	44.79 <sup>b</sup>	49.88 <sup>b</sup>
Greenhouse	FR0	27.03 <sup>a</sup>	27.89 <sup>a</sup>	28.59 <sup>a</sup>	28.78 <sup>ab</sup>	28.96 <sup>b</sup>	29.18 <sup>d</sup>	30.91 <sup>c</sup>	35.93 <sup>c</sup>	41.06 <sup>c</sup>	44.66 <sup>c</sup>
	FR5	27.44 <sup>a</sup>	28.15 <sup>a</sup>	29.02 <sup>a</sup>	29.24 <sup>ab</sup>	29.82 <sup>b</sup>	31.16 <sup>cd</sup>	39.04 <sup>b</sup>	48.10 <sup>b</sup>	51.89 <sup>b</sup>	56.18 <sup>b</sup>
	FR10	28.20 <sup>a</sup>	28.28 <sup>a</sup>	28.83 <sup>a</sup>	29.23 <sup>ab</sup>	29.59 <sup>b</sup>	34.64 <sup>bcd</sup>	44.35 <sup>ab</sup>	54.21 <sup>ab</sup>	57.43 <sup>ab</sup>	62.38 <sup>ab</sup>
	FR15	26.05 <sup>a</sup>	28.25 <sup>a</sup>	29.14 <sup>a</sup>	30.28 <sup>ab</sup>	32.68 <sup>ab</sup>	39.15 <sup>ab</sup>	50.19 <sup>a</sup>	58.01 <sup>a</sup>	61.76 <sup>a</sup>	66.26 <sup>a</sup>
	FR20	25.73 <sup>a</sup>	25.98 <sup>a</sup>	26.56 <sup>a</sup>	27.70 <sup>b</sup>	30.89 <sup>ab</sup>	38.56 <sup>ab</sup>	46.79 <sup>a</sup>	55.29 <sup>a</sup>	58.91 <sup>a</sup>	63.39 <sup>a</sup>
	PM15	24.65 <sup>a</sup>	27.08 <sup>a</sup>	29.14 <sup>a</sup>	31.73 <sup>a</sup>	33.89 <sup>a</sup>	42.30 <sup>a</sup>	50.60 <sup>a</sup>	57.40 <sup>a</sup>	61.29 <sup>a</sup>	64.73 <sup>a</sup>
	CM15	25.01 <sup>a</sup>	27.80 <sup>a</sup>	28.49 <sup>a</sup>	28.70 <sup>ab</sup>	29.38 <sup>b</sup>	35.51 <sup>bc</sup>	46.05 <sup>ab</sup>	52.84 <sup>ab</sup>	56.61 <sup>ab</sup>	61.09 <sup>ab</sup>

Notes: Mean values in a column followed with the same superscript letters are not significantly different according to the least significant difference (LSD) test at  $P < 0.05$ .

**Table 3. Effect of Treatments on Crop Growth Rate (mm)**

Experiment	Treatment	Interval Between Days After Transplanting (DAT)						
		7-21	21-35	35-49	49-63	7-35	35-63	7-63
Field	FR0	1.33 <sup>a</sup>	0.56 <sup>b</sup>	4.43 <sup>b</sup>	8.57 <sup>a</sup>	0.95 <sup>ab</sup>	6.50 <sup>cd</sup>	3.72 <sup>b</sup>
	FR5	1.01 <sup>a</sup>	0.71 <sup>ab</sup>	7.75 <sup>ab</sup>	9.25 <sup>a</sup>	0.86 <sup>ab</sup>	8.50 <sup>ab</sup>	4.68 <sup>ab</sup>
	FR10	1.14 <sup>a</sup>	1.42 <sup>ab</sup>	7.89 <sup>ab</sup>	7.62 <sup>a</sup>	1.28 <sup>ab</sup>	7.75 <sup>abcd</sup>	4.52 <sup>ab</sup>
	FR15	0.90 <sup>a</sup>	2.69 <sup>a</sup>	9.64 <sup>a</sup>	8.86 <sup>a</sup>	1.79 <sup>ab</sup>	9.25 <sup>a</sup>	5.52 <sup>a</sup>
	FR20	0.79 <sup>a</sup>	2.02 <sup>ab</sup>	6.67 <sup>ab</sup>	9.87 <sup>a</sup>	1.41 <sup>ab</sup>	8.27 <sup>abc</sup>	4.84 <sup>ab</sup>
	PM15	0.66 <sup>a</sup>	0.58 <sup>b</sup>	4.79 <sup>b</sup>	9.06 <sup>a</sup>	0.62 <sup>b</sup>	6.93 <sup>bcd</sup>	3.77 <sup>b</sup>
	CM15	1.70 <sup>a</sup>	2.35 <sup>ab</sup>	4.64 <sup>b</sup>	7.38 <sup>a</sup>	2.02 <sup>a</sup>	6.01 <sup>d</sup>	4.02 <sup>b</sup>
Greenhouse	FR0	1.11 <sup>bc</sup>	0.27 <sup>b</sup>	1.39 <sup>c</sup>	7.25 <sup>a</sup>	0.69 <sup>cd</sup>	4.32 <sup>c</sup>	2.51 <sup>d</sup>
	FR5	1.17 <sup>bc</sup>	0.57 <sup>b</sup>	6.58 <sup>b</sup>	9.18 <sup>a</sup>	0.85 <sup>cd</sup>	7.88 <sup>b</sup>	4.37 <sup>c</sup>
	FR10	0.45 <sup>c</sup>	0.54 <sup>b</sup>	10.54 <sup>ab</sup>	9.34 <sup>a</sup>	0.50 <sup>d</sup>	9.94 <sup>ab</sup>	5.22 <sup>bc</sup>
	FR15	2.21 <sup>abc</sup>	2.53 <sup>a</sup>	12.51 <sup>a</sup>	8.27 <sup>a</sup>	2.37 <sup>ab</sup>	10.39 <sup>a</sup>	6.38 <sup>ab</sup>
	FR20	0.64 <sup>bc</sup>	3.09 <sup>a</sup>	11.36 <sup>ab</sup>	8.66 <sup>a</sup>	1.84 <sup>bc</sup>	10.01 <sup>ab</sup>	5.93 <sup>ab</sup>
	PM15	3.21 <sup>a</sup>	3.39 <sup>a</sup>	11.94 <sup>a</sup>	7.63 <sup>a</sup>	3.30 <sup>a</sup>	9.79 <sup>ab</sup>	6.54 <sup>a</sup>
	CM15	2.48 <sup>ab</sup>	0.65 <sup>b</sup>	11.91 <sup>a</sup>	7.54 <sup>a</sup>	1.56 <sup>bc</sup>	9.73 <sup>ab</sup>	5.64 <sup>abc</sup>

Notes: Mean values in a column followed with the same superscript letters are not significantly different according to the least significant difference (LSD) test at  $P < 0.05$ .

levels of dry matter accumulation in both the field and greenhouse experiments. In the greenhouse experiment, plants treated with FR10, FR20, PM15 and CM15 displayed a DWT similar to that of plants treated with FR15. Plants in the control in both the field and greenhouse experiments displayed the lowest biomass accumulation.

### 3.3.4 Effects of Treatments on the RSR

The highest RSR was observed in the control in both the

field and greenhouse experiments, with FR15 consistently displaying a relatively low RSR (Table 5). In the greenhouse experiment, there were no significant differences in RSR among FR10, FR15, FR20, PM15 and CM15 treatments.

### 3.4 Effect of Treatments on Physicochemical Properties of the Soil after Harvesting

Manure treatments, irrespective of their source, significantly influenced the physicochemical properties

**Table 4. Effect of Treatments on Number of Leaves per Plant**

Treatment/DAT	Days After Transplanting (DAT)					
	Field			Greenhouse		
	28	49	70	28	49	70
FR0	4.17 <sup>c</sup>	6.00 <sup>a</sup>	8.25 <sup>a</sup>	4.25 <sup>b</sup>	6.25 <sup>ab</sup>	7.38 <sup>b</sup>
FR5	4.33 <sup>bc</sup>	6.33 <sup>a</sup>	8.42 <sup>a</sup>	4.50 <sup>b</sup>	6.46 <sup>ab</sup>	7.75 <sup>ab</sup>
FR10	4.50 <sup>abc</sup>	6.33 <sup>a</sup>	8.08 <sup>a</sup>	4.62 <sup>b</sup>	6.48 <sup>ab</sup>	7.88 <sup>ab</sup>
FR15	4.83 <sup>ab</sup>	6.67 <sup>a</sup>	8.33 <sup>a</sup>	4.50 <sup>b</sup>	7.00 <sup>a</sup>	8.38 <sup>a</sup>
FR20	4.83 <sup>ab</sup>	6.33 <sup>a</sup>	7.83 <sup>a</sup>	4.60 <sup>ab</sup>	7.00 <sup>a</sup>	8.38 <sup>a</sup>
PM15	5.00 <sup>a</sup>	6.67 <sup>a</sup>	8.00 <sup>a</sup>	5.25 <sup>a</sup>	6.75 <sup>ab</sup>	8.38 <sup>a</sup>
CM15	4.67 <sup>abc</sup>	7.00 <sup>a</sup>	8.08 <sup>a</sup>	4.38 <sup>b</sup>	6.18 <sup>b</sup>	7.50 <sup>b</sup>

Notes: Mean values in a column followed with the same superscript letters are not significantly different according to the least significant difference (LSD) test at  $P < 0.05$ .

**Table 5. Effect of Treatments on Plant Fresh Weight, Dry Weight and Root to Shoot Ratio**

Treatment	Field Experiment			Greenhouse Experiment		
	FWT	DWT	RSR	FWT	DWT	RSR
FR0	35.12 <sup>ab</sup>	2.82 <sup>c</sup>	0.283 <sup>a</sup>	19.87 <sup>d</sup>	2.24 <sup>c</sup>	0.306 <sup>a</sup>
FR5	48.02 <sup>ab</sup>	3.80 <sup>abc</sup>	0.236 <sup>ab</sup>	31.84 <sup>c</sup>	3.17 <sup>bc</sup>	0.267 <sup>ab</sup>
FR10	44.56 <sup>ab</sup>	3.40 <sup>bc</sup>	0.271 <sup>a</sup>	40.68 <sup>b</sup>	3.94 <sup>ab</sup>	0.172 <sup>c</sup>
FR15	56.84 <sup>a</sup>	4.87 <sup>a</sup>	0.178 <sup>b</sup>	49.06 <sup>a</sup>	4.25 <sup>a</sup>	0.196 <sup>bc</sup>
FR20	43.10 <sup>ab</sup>	3.15 <sup>bc</sup>	0.224 <sup>ab</sup>	46.56 <sup>ab</sup>	3.93 <sup>ab</sup>	0.179 <sup>c</sup>
PM15	33.15 <sup>b</sup>	3.13 <sup>bc</sup>	0.215 <sup>ab</sup>	47.27 <sup>ab</sup>	4.21 <sup>a</sup>	0.180 <sup>c</sup>
CM15	46.16 <sup>ab</sup>	4.12 <sup>ab</sup>	0.228 <sup>ab</sup>	40.85 <sup>b</sup>	3.67 <sup>ab</sup>	0.211 <sup>bc</sup>

Notes: Mean values in a column followed with the same superscript letters are not significantly different according to the least significant difference (LSD) test at  $P < 0.05$ .

of post-harvest soils in both the field and greenhouse experiments (Table 6). Frass, PM and CM significantly decreased the soil pH. Relatively lower pH values were observed in the post-harvest period compared with the initial soil status with exceptions of FR5 and FR0 in the field experiment and FR5 in the greenhouse experiment. The lowest pH was observed in FR15 and FR20 for the field experiment and FR20 and CM15 for the greenhouse experiment. OC percentage in soils was highly influenced by the addition of manures. The maximum percentage OC was recorded in PM15, FR10, and FR20 for the field experiment, and FR20 for the greenhouse experiment. In both cases, they were at parity with the initial soil status but statistically higher than the control treatment (FR0) in the post-harvest period. Low OC percentages were observed in both FR0 and FR5.

Addition of manures and frass to the soil also influenced levels of essential nutrients such as N, P and K in comparison to the control (Table 6). Treatments CM15, PM15, FR20, and FR15 gave the highest available N in both the field and greenhouse experiments. All frass treatments had lower N levels compared to the initial soils in the field experiment. FR0 had the lowest concentration of N in post-

harvest soils in both experiments. FR20 had statistically higher available P, while the control treatment had the lowest. There were significantly higher concentrations of P in the post-harvest soils for all treatments relative to the initial soils except in the field experiment where FR0, FR15, CM and FR20 had lower concentrations. The highest concentration of K was found in FR20, closely followed by FR15 in both field and greenhouse experiments. All the frass treatments had relatively higher concentrations of K in the post-harvest soils compared to the control and the CM and PM treatments. Frass also exhibited relatively higher amounts of exchangeable cations such as Mg and calcium. Overall, all treatments improved soil nutrient status, confirming that the use of organic manures can increase the efficiency with which nutrients are used.

## 4 DISCUSSION

### 4.1 Plant Growth and Development

No significant differences in plant height were observed in the first few weeks after transplanting in both the field and greenhouse experiments. This was likely due to establishment of the transplants and gradual mineralization of organic N fractions of manures. Onions have characteristic shallow rooting and may take time to

**Table 6. Effect of Treatments on Soil Physicochemical Properties**

Experiment	Treatment	EC (µS/M)	pH	OC (%)	P (ppm)	N (%)	K (ppm)	Ca (ppm)	Mg (ppm)
Field	Soil*	7.65 <sup>c</sup>	8.57 <sup>a</sup>	1.26 <sup>a</sup>	374 <sup>d</sup>	0.316 <sup>c</sup>	536 <sup>e</sup>	11329 <sup>a</sup>	339 <sup>g</sup>
	FR0	5.00 <sup>f</sup>	7.85 <sup>e</sup>	1.16 <sup>bc</sup>	331 <sup>e</sup>	0.045 <sup>h</sup>	529 <sup>e</sup>	6861 <sup>g</sup>	373 <sup>f</sup>
	FR5	5.20 <sup>f</sup>	8.65 <sup>a</sup>	1.05 <sup>d</sup>	472 <sup>b</sup>	0.068 <sup>f</sup>	641 <sup>b</sup>	6180 <sup>h</sup>	122 <sup>h</sup>
	FR10	5.80 <sup>e</sup>	8.19 <sup>c</sup>	1.29 <sup>a</sup>	499 <sup>a</sup>	0.049 <sup>g</sup>	589 <sup>c</sup>	8087 <sup>b</sup>	435 <sup>c</sup>
	FR15	6.80 <sup>d</sup>	7.80 <sup>e</sup>	1.23 <sup>ab</sup>	244 <sup>f</sup>	0.106 <sup>e</sup>	646 <sup>b</sup>	7914 <sup>c</sup>	505 <sup>b</sup>
	FR20	3.30 <sup>g</sup>	7.47 <sup>f</sup>	1.29 <sup>a</sup>	147 <sup>h</sup>	0.206 <sup>d</sup>	712 <sup>a</sup>	7374 <sup>d</sup>	546 <sup>a</sup>
	PM15	10.90 <sup>b</sup>	8.06 <sup>d</sup>	1.31 <sup>a</sup>	400 <sup>c</sup>	0.339 <sup>b</sup>	561 <sup>d</sup>	7253 <sup>e</sup>	409 <sup>e</sup>
	CM15	27.2 <sup>a</sup>	8.42 <sup>b</sup>	1.11 <sup>cd</sup>	169 <sup>g</sup>	0.465 <sup>a</sup>	472 <sup>f</sup>	7094 <sup>f</sup>	423 <sup>d</sup>
	Soil*	7.65 <sup>b</sup>	8.57 <sup>b</sup>	1.26 <sup>ab</sup>	374 <sup>f</sup>	0.316 <sup>a</sup>	536 <sup>e</sup>	11329 <sup>d</sup>	339 <sup>e</sup>
	FR0	3.10 <sup>h</sup>	8.68 <sup>a</sup>	1.17 <sup>bcd</sup>	431 <sup>e</sup>	0.001 <sup>g</sup>	432 <sup>f</sup>	10241 <sup>g</sup>	321 <sup>g</sup>
Greenhouse	FR5	5.20 <sup>g</sup>	8.63 <sup>a</sup>	0.98 <sup>e</sup>	610 <sup>c</sup>	0.049 <sup>f</sup>	595 <sup>d</sup>	10983 <sup>e</sup>	338 <sup>f</sup>
	FR10	5.90 <sup>f</sup>	8.56 <sup>b</sup>	1.08 <sup>de</sup>	472 <sup>d</sup>	0.049 <sup>f</sup>	671 <sup>b</sup>	12260 <sup>b</sup>	495 <sup>a</sup>
	FR15	7.20 <sup>d</sup>	8.43 <sup>c</sup>	1.14 <sup>cd</sup>	470 <sup>d</sup>	0.050 <sup>e</sup>	730 <sup>a</sup>	11708 <sup>c</sup>	446 <sup>b</sup>
	FR20	8.80 <sup>a</sup>	8.33 <sup>d</sup>	1.36 <sup>a</sup>	769 <sup>a</sup>	0.245 <sup>b</sup>	731 <sup>a</sup>	10476 <sup>f</sup>	439 <sup>c</sup>
	PM15	6.50 <sup>e</sup>	8.48 <sup>c</sup>	1.18 <sup>bcd</sup>	674 <sup>b</sup>	0.128 <sup>d</sup>	631 <sup>c</sup>	13182 <sup>a</sup>	409 <sup>d</sup>
	CM15	7.40 <sup>c</sup>	8.13 <sup>e</sup>	1.23 <sup>bc</sup>	433 <sup>e</sup>	0.130 <sup>c</sup>	400 <sup>g</sup>	9838 <sup>h</sup>	316 <sup>h</sup>

Notes: \*Soil represents the soil samples taken before the start of the experiment; EC: Electrical conductivity; OC: Organic carbon; P: Available phosphorus; N: Total Nitrogen; K: Exchangeable Potassium; Ca: Calcium; Mg: Magnesium. Mean values in a column followed with the same superscript letters are not significantly different according to the least significant difference (LSD) test at  $P < 0.05$ .

establish, with maximum nutrient uptake occurring within 15 DAT to 60 DAT<sup>[29,33]</sup>. Additionally, insect frass has high levels of labile OC and bound ammonium N<sup>[11-14,22]</sup> that would need time to be mobilized to forms of nitrates that are available to plants during the nitrification process. Environmental factors such as temperature, moisture and soil conditions could also have led to the slow rate of N release since they affect soil microbes and dictate immobilization and denitrification pathways<sup>[13,17]</sup>.

All manure treatments, frass inclusive, had significantly higher plant heights and growth rates than the control treatment throughout the experimental period. This illustrated the availability and ease of uptake of nutrients from frass while simultaneously demonstrating the soils natural deficiency to support onion growth<sup>[34]</sup>. Our study confirmed previous reports<sup>[16,22,24]</sup> that nutrients in frass are more labile and readily mineralized as shown by the consistency within which high application rates of FR15 and FR20 contributed to either relatively high growth rates and plant heights or were at parity with conventional manures in both experiments. The high amounts of soluble C fraction in the frass could have stimulated growth of the frass microbial population, thus resulting in a high rate of C and N mineralization<sup>[12]</sup>. Cricket frass at all application rates released essential minerals more rapidly but consistently to supply crop growth needs, albeit more efficiently than PM and CM. This is consistent with the study conducted by Houben et al.<sup>[14]</sup>, who found that frass released nutrients

homogeneously due to uniform distribution of nutrients within the frass organic matter, which reduces the absence of isolated mineral phases. The better performance of plants in frass treatments relative to that of plants under the conventional manures could also be linked to presence of plant growth promoters which have been shown to be abundant in manure obtained from insects<sup>[16]</sup>. The observed inconsistency in the height of plants under PM and CM between the field and greenhouse experiments suggests that frass was more stable and least susceptible to environmental influence, which probably led to higher losses of minerals from conventional manures through leaching and/or volatilization in the field. The higher number of leaves displayed by plants in FR15 and FR20 could be attributed to the high N and P concentrations in the frass biofertilizer that promoted cell division, resulting in rapid growth of the shoots. Nutrients in the frass could also have facilitated the use of other essential elements in the soil. This was consistent with previous findings<sup>[34]</sup> that reported significant differences in plots treated with CM in comparison to poultry and sheep manure on bulb onions under irrigation. Bua et al.<sup>[35]</sup> also reported significant differences in leaves when green manure, compost and farmyard manure were used on bulb onions variety red creole in Uganda.

#### 4.2 Yield Attributes

The control treatment consistently exhibited the highest root to RSR in both experiments, suggesting an inherent stress within the plants that did not receive manure

treatments. Longer roots would develop to mobilize soil nutrients especially when nutrients are limited, leading to higher root to RSRs. Root to RSR has been suggested as a sensitive indicator of plant stress induced by chemical or physical agents<sup>[36]</sup>. However, different plants are known to respond uniquely to a specific stimulus<sup>[37]</sup> hence it may not be an obvious or direct indicator as alluded. The manure treatments, frass inclusive, supplied sufficient nutrients to the plants leading to the accumulation of biomass above the ground.

Plants in the control treatment performed poorly in both the field and greenhouse experiments with respect to dry matter accumulation, an indication of the importance of nutrients for boosting onion yields. Organic matter is known to buffer soil properties<sup>[10,38,39]</sup> and as such, manure treated plots may have improved soil porosity and water holding capacity that enabled root penetration and ease of nutrient absorption. Frass was more effective in improving onion growth and biomass accumulation than PM and CM respectively. The higher N content in frass manure promoted succulence in the onion leaves, resulting in higher FWTs. The rapid vegetative growth observed in frass treatments may have resulted in improved interception of photosynthetically active radiations hence accumulation of more assimilates in the pseudo-bulbs of spring onions. Yassen and Khalid<sup>[40]</sup> previously associated N with rapid leaf growth, which in turn improved the leaf surface area available for interception of photosynthetically active radiations. The considerable quantity of P in the frass biofertilizer could have also resulted in rapid growth of roots which enhanced nutrient absorption leading to a high concentration of assimilates in the plants. These findings are in accord with a previous study<sup>[22]</sup> in which addition of N-rich frass from cabbage army worm (*Mamestra brassicae*) significantly increased biomass of *Brassica rapa* plants compared to the control with no treatment. Similarly, the study observed an increase in plant biomass with a unit increase in amounts of frass applied an indication of a threshold in insect frass loadings that affect growth.

Phytohormones and microorganisms in organic manures are known to stimulate plant growth and nutrient uptake. Frass, which is rich in carbon and N<sup>[17,40]</sup>, may have facilitated microbial activity and mineralization of nutrients to easily absorbable forms for onion growth. Similar studies have reported higher yields in maize when BSF frass was applied<sup>[41]</sup> to onion plants treated with organic manures<sup>[34,35,42]</sup>. Additionally, frass treatments have been linked to increased tolerance to stresses such as drought, pests and diseases<sup>[14,22]</sup>. Chitosan arising from crickets and contained in frass have been implicated in stimulating resistance to disease<sup>[20]</sup>. Chitosan is documented to have antimicrobial activity against bacterial and fungal pathogens<sup>[11]</sup>, and thus could have also contributed to the improved overall performance of the onion plants leading

to higher yields compared to PM and CM. The comparable effects of frass on growth and biomass accumulation in this study is an indication that cricket frass is a potential nutrient source and therefore could be used as an alternative to conventional manures.

### 4.3 Soil Physicochemical Properties

Although the pH of the soil treated with frass was not as low as that of the soil treated with CM, the application of frass significantly decreased the pH of post-harvest soils (Table 6). FR15 and FR20 plots in the field experiment and FR20 and CM15 in the greenhouse experiment had the lowest pH values. This could be due to rapid decomposition of the organic fraction of manure leading to production of CO<sub>2</sub> and organic acids that lowered the soil pH. Similar findings have been reported<sup>[10,14,43]</sup>, highlighting the role played by organic manure in buffering soil pH regardless of its nature. Application of organic manures slightly increased the soil's OC content, while the control treatment maintained a significantly low OC content. Addition of organic residue is known to increase the soil's OC content. However, the increase is usually dependent on the incubation period and as such, the OC percentage in soils may initially increase and then decrease to a constant value when less resistant fractions have been broken down<sup>[44-48]</sup>. Frass has a short incubation period<sup>[16]</sup>, and was probably mineralized more rapidly and used within the first few days explaining the similarity in results with that of the control.

With regards to the nutrient status of post-harvest soils, frass, CM and PM all improved P and K contents of the soils. This affirms the enhanced and efficient use of nutrients when manures are used. Manure treatments, frass inclusive, improved the P status in soil albeit more pronounced in the greenhouse experiment where all treatments had higher residual P than the control and initial soils. K concentration in the frass treated soils was also significantly higher than the control and initial soil. N from manure treated soils was consistently lower in the post-harvest soils compared to the initial soils but higher than that of the control. This could be attributed to high uptake of nitrates by the rapidly growing onions. The decrease in extractable N from manure treatments could be a result of microbial immobilization and denitrification processes<sup>[12]</sup>, as microbes tend to compete favorably with plants for the use of N especially from organic sources such as frass. Only the top 0-20 cm depth soil was sampled in this study, and as such, it could not account for N losses through leaching and or ammonium volatilization<sup>[5,12]</sup>. Nevertheless, manure treatments, irrespective of the source, had higher residual N than the control, signifying the ease of release of N from such sources in improving soil nutrient availability and growth and yield of crops<sup>[22]</sup>.

The findings in this study are similar to those of a previous study<sup>[44]</sup>, which stated that the addition of



organic manures improves the soil's physicochemical properties, and that this may have a direct or indirect effect on plant growth and yields. Therefore, the increased OC and essential nutrients arising from frass application improved performance of the spring onion as well as soil characteristics in this study. Organic amendment might have elicited microorganism activity and nutrient availability more than the control, accounting for the higher yields<sup>[45]</sup>. Growth performance and residual effects of frass on the soil seemed to have increased with an increase in rates of application, illustrating the need to consider both economic and social implications in the short and long term if a choice has to be made on which application rate to use. Plants in FR15 outperformed plants in the CM and PM, and this could be due to availability of sufficient nutrients for uptake by the onion plants<sup>[46]</sup>.

## 5 CONCLUSION

There is limited data regarding biofertilizer derived from insect frass and its usage in agriculture amidst the intensive global interest in exploiting insects as sources of nutrition for humans as well as livestock reaffirms biofertilizer derived from cricket waste as having the potential to improve soil fertility and enhance the growth and yield of onion. 15t/Ha of frass-derived manure had positive residual effects on the soil's physicochemical properties, which in turn contributed to improved growth and yield parameters of spring onions. Manure from frass is relatively stable and not prone to leaching and volatilization as compared to either PM or CM, thus making insect frass-derived bio-fertilizer a potential alternative for nutrient recycling and sustainable agriculture especially in poorer regions of the world such as the Sub-Saharan Africa, where farmers have limited resources and soil degradation occurs at an alarming rate. An integrated insect-crop farming presents a perfect synchrony through which food insecurity can be alleviated via backyard farms and sustainable peri-urban agro-food systems which require less space but produce safer foods for daily household consumption. Therefore, edible insects such as crickets are potential sources of stable biofertilizers for upscaling and commercialization of agricultural productivity, while at the same time providing proteins for resource poor farmers in marginal environments. Use of frass from edible insects as a biofertilizer is also consistent with the principles of a circular economy which promotes the re-introduction of valuable materials into the food production chain as opposed to linear models that favor unidirectional processes where products end up as disposable waste. More studies are needed to improve the current knowledge on the use of insect frass as a biofertilizer. Future research should focus on the effects of cricket frass on quality-related parameters such as nutrient, mineral, proteins and vitamin contents in crops using varying frass manure amendments in different soil types.

## Acknowledgements

This project was funded by the World Bank through the

Africa Center of Excellence in Sustainable Use of Insects as Food and Feeds (INSEFOODS) of Jaramogi Oginga Odinga University of Science and Technology.

## Conflicts of Interest

The authors declared that they have no competing interests. The World Bank had no role in the design of the study; collection, analysis or interpretation of data; in the writing of the manuscript or in the decision to publish the results.

## Author Contribution

Ogaji SO designed and performed the experiments, analyzed the data, and drafted the manuscript. Watako AO and Nyongesah JM supervised the experiments to completion and revised the manuscript. Bulli P contributed to data analysis, interpretation, and revised the manuscript. All authors read and approved the final version of the manuscript.

## Abbreviation List

Ca, Calcium  
CEC, Cation exchange capacity  
CGR, Crop growth rate  
CM, Cattle manure  
CM15, 15t/ha of cattle manure  
DAT, Days after transplanting  
DWT, Dry weight measurement  
FR0, Control of zero organic fertilizer application  
FR10, 10t/ha of frass  
FR15, 15t/ha of frass  
FR20, 20t/ha of frass  
FR5, 5t/ha of frass  
FWT, Fresh weight  
JOOUST, Jaramogi Oginga Odinga University of Science and Technology  
K, Potassium  
Mg, Magnesium  
N, Nitrogen  
Na, Sodium  
NLF, Number of leaves per plant  
OC, Organic carbon  
P, Phosphorus  
PM, Poultry manure  
PM15, 15t/ha of poultry manure  
RSR, Root to shoot ratio

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