Rabbit urine as a biopesticide and biofertilizer

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Abstract

The increased demand for food production has created higher needs for crop nutrients and protection. Although synthetic fertilizers and pesticides have increased food security and ensured yield for farmers worldwide, there are growing concerns about the environmental implications of over reliance of these fertilizers and pesticides as well as concern for pest resistance due to persistent pesticide use. This study sought to evaluate alternatives towards soil nutrition and pest control. This experiment evaluated wether or not 50% diluted rabbit urine can be used as a bio fertilizer and/or biopesticide as a sustainable and affordable alternative. The experiment used kale also known as collards, *Brassica oleracea* var. acephala D.C and aphids (Homoptera: Aphididae) as test subjects. There was no statistical difference between pesticidal control experiments and urine sprayed experiments in number of aphids. The number of leaves and dry matter weight was statistically higher in rabbit urine treated experiments compared to negative control. We therefore conclude from this experiment that there was evidence that rabbit urine can be used as biofertilizer but not e as a pesticide aginst aphids. Given that we diluted rabbit urine in 1:1 with water, further investigations could be undertaken to evaluate higher concentrations of urine as well as combine rabbit urine with other pesticides such as neem oil. .

Keywords: Rabbit urine, biopesticide, biofertilizer, pest control, plant nutrition

Ikisiri

Ongezeko la mahitaji ya uzalishaji wa chakula limesababisha mahitaji ya juu ya virutubisho pamoja na ulinzi wa mazao ya mimea. Ingawa mbolea sanisi na dawa za kuuwa wadudu zimeongezea usalama wa chakula na kuhakikisha mavuno kwa wakulima duinani kote, kuna ongezeko la wasiwasi kuhusiana na athari za kimazingira za mbolea hizi na dawa hizi za kuulia wadudu pamoja na upinzani wa wadudu kutokana na uzoefu wa utumiaji uliokithiri wa dawa hizi. Jaribio ili lilitathimini kama au la, asilimia hamsini ya mkojo wa sungura uliozimuliwa unaweza kutumika kama mbolea ya kibiolojia na/ au dawa ya kuuwa wadudu kama mbadala endelevu na nafuu kwa mbolea sanisi na dawa za kuulia wadudu. Jaribio hili lilitumia mboga za kale na wadudu wa *aphids* kama vigezo vya majaribio. Kulikuwa na ushahidi wa wastani ambao ulionyesha kwamba mkojo wa sungura unaweza kutumika kama mbolea ya mimea lakini hakukuwa na ushahidi wowote wa kuonyesha kuwa mkojo wa sungura unaweza kutumika kama dawa dhidi ya wadudu wa *aphids*. Ili kchunguza zaidi athari ya mkojo wa sungura kama dawa ya wadudu, kuna haja ya kutathmini viwango tofauti vya mkusanyiko wa mkojo pamoja na kuchanganya mkojo wa sungura na dawa nyingine za kuuwa wadudu kama vile mafuta ya mwarobaini.

Msamiati maalum: Mkojo wa sungura, dawa ya kuua wadudu, mbolea ya mimea, udhibiti wa wadudu, lishe ya mimea

Table of contents

Abbreviations

SLU	Swedish University of Agricultural Sciences
CAN	Calcium ammonium nitrate, $CaxH_3NxHNO_3$ (fertilizer)
DAP	Diammonium phosphate, $(NH_4)_2(HPO_4)$ (fertilizer)
PB-test	Parametric Bootstrap test

1. Introduction

The demand for increased food production due to increased world population created higher needs for crop nutrients and crop protection. Chemical fertilizers created through the Haber-Bosch process made it possible to intensify agricultural practices in the 1900s to increase yields, and chemical pesticides have provided farmers with an increased security of yield as well as food security for many people (Smil 2001). However, the production of chemical fertilizers is very energy demanding and with the current climate change crisis due to greenhouse gas emissions the sustainability of these processes are increasingly questioned.

In Kenya, rabbits have become more prevalent as livestock in recent years, and rabbit urine is a valuable agricultural resource that could be utilized as a biofertilizer or even as a biopesticide (Kemunto et. al, 2022; Mutai 2020). Smallhold farmers in Kenya use rabbit urine as both a fertilizer and a biopesticide as it is readily available and affordable. However, there is little scientific evidence that rabbit urine as a biopesticide is an effective method (Kemunto et. al, 2022; Mutai 2020).

Synthetic nitrogen fertilizers emitted 1.13 Gt CO_2e (giga tonnes of CO_2 equivalent) in 2018, which is about 2.1% of global greenhouse gas emissions (Menegat et. al 2022). Organic nitrogen fertilizers do not need the large amounts of energy needed for production, as organic nitrogen is found in livestock waste and green manure. Utilizing organic nitrogen, which is often considered waste or a byproduct on farms, can play a crucial role in mitigating harmful greenhouse gas emissions. In addition, this approach can help farmers reduce costs and improve environmental and economic sustainability in the long run. When biofertilizers from livestock are used, the nutrients are recycled and better utilized locally, instead of repeated input of synthetic mineral fertilizers which increase the risk of nutrient leaching and runoff.

Chemical pesticides can have detrimental effects on the environment and local fauna, particularly pollinating insects and aquatic organisms. According to Hallman et al. (2017), the use of pesticides plays a significant role in the global decline of biodiversity. In a study of 30 fields in Germany, organic fields were found to have five times more plant species and approximately twenty times more pollinator species than conventionally treated fields (Hallman et. al). Certain pesticides, such as DDT, can bioaccumulate and harm organisms higher up in the food chain, while others like organophosphates can be toxic to aquatic vertebrates (Heinrich-Böll-Stiftung 2022). Neonicotinoids and some pyrethroids are harmful to bees and other beneficial insects that provide vital ecosystem services like pollination and natural pest control (Heinrich-Böll-Stiftung 2022). The excessive use of pesticides may also lead to pest resistance, rendering many compounds ineffective in the long run. Moreover, some pesticides can pose health risks to humans due to trace amounts in food and unsafe handling during application (Heinrich-Böll-Stiftung 2022).

In light of the environmental concerns associated with chemical pesticides and fertilizers, it is important to explore sustainable alternatives like biofertilizers and biopesticides, such as animal wastes and allelochemicals. However, the effectiveness of some ofthese alternatives compared to their synthetic counterparts remains unclear. To address this, I investigated the impact of rabbit urine as a biofertilizer and biopesticide on kale crops. The objective of this study was to determine whether rabbit urine can be a viable substitute or supplement for commercial fertilizers and pesticides while promoting environmental sustainability

Kale, *Brassica oleracea* Var. Acephala D.C*,* commonly referred as Sukuma wiki in Kenya, has traditionally been an important crop for low income earning families in East Africa. The literal translation of Sukuma wiki is “push the week” or “stretch the week”, as the vegetable is relatively affordable and is consumed in almost every meal by resource poor families. To keep the cost of this culturally important crop low, the cost of agricultural inputs also has to stay low while still ensuring a sufficient harvest. Synthetic fertilizers and pesticides can be very expensive and thus raising the cost of Sukuma wiki production during times when pest populations are large and soil nutrient conditions are subpar. In order to reduce costs, farmers use readily available and affordable resources for crop protection and nutrition. The use of livestock wastes, and in particular urine, is one of the alternatives.

1.1.1 Rabbit urine as a biofertilizer

Nitrogen is a crucial part of plant nutrition, and is the plant nutrient that is required in the largest amount. Nitrogen plays an important role in photosynthesis as it is required in chlorophylls. Photosynthesis is the mechanism through which plants grow and build CO_2 into organic compounds, which means that sufficient nitrogen availability in the soil is essential for plant growth. The plant roots take up nitrogen in the forms of ammonium (NH_4^+), nitrate (NO_3^-), amino acids and urea (CH_4N_2O). Nitrate has to be reduced to ammonium to be able to be built into organic matter in the plant (Marshner 2012). Cattle urine contains large amounts of nitrogen mostly in the form of ammonium and urea (Miah et. al 2017).

1.1.2 Urine as a biopesticide

Livestock urine could prove effective against some crop pests. A study investigated the bioefficacy of cow urine and different bio-pesticides against sucking insect pests in cotton and found that undiluted cow urine by itself as well as in combination with different types of bio-pesticides proved effective against pests like aphids, jassids and thrips and increased yield (Singh et. al, 2019). Another study investigated the effect of rabbit urine on the fall army worm in which they found that rabbit urine led to reduced larval feeding and therefore increased mortality, as well as reduced egg hatchability (Kemunto et. al, 2022).

1.2 Aphids

Aphids are sap sucking insects in the order Hemiptera and family Aphidoidea. They are mostly sedentary as they attach with their mouth proboscis in the phloem tissue of the plant to ingest the sap. They cause direct and indirect damage through transmission of plant diseases. Symptoms of aphid infestation include curling of the leaves (this also gives the aphids extra protection) and yellowing (chlorosis) of the leaves and stunted or abnormal growth (CABI, 2022; Pal, 2013; Figure 1).

Figure 1:Typical leaf-curl damage caused by aphids on kale. Photo: Kari Løe.

The aphids used for this project were the cabbage aphid *Brevicoryne brassicae* (Linnaeus, 1758) Hemiptera: Aphididae (Figure 2) which is uses *Brassica* plants as hosts and the green peach aphid *Myzus persicae* (Linnaeus, 1758) Hemiptera: Aphididae (Figure 3), which is a generalist species who thrive on many different host plants. Both of these aphids are commonly found on *Brassicae* plants and cause significant yield losses. The cabbage aphid is easily distinguishable from the peach aphid as it has a grey wax coating that the peach aphid does not. In warm climates aphid populations only consist of females as only colder temperatures induce the production males. Generation times are therefore very short in warmer climates because the wingless females give birth to live wingless nymphs when males are not present (CABI 2022, Pal 2013).

Figure 2: A colony of Brevicoryne brassicae and some accumulated wax on kale. Photo: Kari Løe.

Figure 3: A colony of green Myzus persicae on kale. Photo: Kari Løe

1.3 Objective and hypotheses

1.3.1 Objective

The main objective of this study was to evaluate rabbit urine as a biofertilizer and biopesticide as a low cost alternative to commercial fertilizers and insecicide against aphids on kale.

Specific objectives were to:

- evaluate rabbit urine as a fertilizer in kale, Brassica oleracea var. Acephala D.C.growth.
- evaluate rabbit urine as an insecticide towards aphids on kale

1.3.2 Hypotheses

Hypothesis 1: Rabbit urine has comparable nutritional potential as commercial CAN fertilizer in kale growth/ production.
Hypothesis 2: Rabbit urine has comparable effect as conventional insectide in reducing aphid population growth in kale production.

2. Methods

2.1 Materials

Kale, *Brassica olearacea* var. acephala D.C. plants of the F_1 Mfalme variety purchased as seedlings from a local plant nursery in Embu, Kenya were used in experiments. The pests used abbage aphids, *Brevicoryne brassicae* and peach aphids, *Myzus persicae* as both species use kale as hosts. The aphids were collected from kale plants from local farms. The commercial fertilizer and insectidcide used for the experiment were a kind gift from the university of Embu farm unit. The fertilizer used in the experiment was a calcium ammonium nitrate, CAN, fertilizer with 27% nitrogen. The insecticide used was Rapid 120 EC (Amiran) which contains the active ingredients Acetamiprid 100 g/L (neonicotinoid) and Emamectin Benzoate 20 g/L (avermectin). The rabbit urine was sourced from a local rabbit farm. Two litres of urine was collected from four rabbits over the course of one week.

2.2 Experimental methods

The experiment contained two factors to be investigated; a nutritional factor and a pesticidal factor. The nutritional factor contained 3 treatments applied in the soil; negative control (water without added nutrients), positive control (CAN fertilizer, calcium ammonium nitrate) and 50% diluted rabbit urine. The pesticidal factor contained 3 foliar spray treatments; negative control (water), positive control (l synthetic insecticide) and rabbit urine. In total there were 9 combinations of treatments (Table 1), each with fve (5) replicates. A total of 45 plants were used in the experiment.

Table 1: The different treatmentsused to test kale productivity and aphid population growth

Treatment	**Nutrient**	**Pesticide**
1	Water	Water
2	Water	Rabbit urine
3	Water	CAN

4	Rabbit urine	Water
5	Rabbit urine	Rabbit urine
6	Rabbit urine	CAN
7	CAN	Water
8	CAN	Rabbit urine
9	CAN	CAN

A sample chemical analysis of the chemical properties of the rabbit urine was carried out on the first batch at the University of Embu chemistry lab.

The kale seedlings were bought on the 4th of February 2023 and were 29 days old at the time. They were planted in 2 litre buckets with cocopeat as the substrate and all were supplemented with 20 g of Diammonium phosphate (DAP) fertilizer as cocopeat is a nutrient deficient substrate. The nitrogen content of DAP fertilizer is 18% which made the total amount of nitrogen in 20 g of DAP 3.6g per plant. The seedlings were then watered twice a day for 13 days until the 17th of February 2023 when the nutritional treatment started. The pots were numbered 1-45 and divided into 5 blocks (one for each replicate) and then randomized without substitution within the block to make a randomized block design. Each pot was assigned a treatment. The number of fully opened leaves and dead leaves were counted once a week for a month until the 20th of March 2023 when the experiment was terminated. The dead leaves were collected throughout the experiment to ensure to get the total amount of biomass produced by the plant into the calculation of the dry matter. On the 17th of February 2023 when the nutritional treatments were started, 15 plants were given 200 ml water, 15 plants were given 5 g of CAN fertilizer (+ 200 ml water) and 15 plants were given 100 ml of rabbit urine diluted with 100ml of water (200 ml in total). According to the rabbit urine analysis (table 2) the specific weight of rabbit urine is 1.0133 and the nitrogen content was 1.3 % of the mass. That means that the total nitrogen content of 100 ml of rabbit urine was 1.317 grams. The nitrogen content of the CAN fertilizer was 27% and the total nitrogen in the 5 grams applied to each pot was 1.35 grams. The plants were then watered once a day (to avoid leaching of the nutrients) with 200 ml of water, which was then reduced to 100 ml per day when 200ml proved to be excessive a week after the treatment started.

On 13th of February 2023 it was observed that two of the pots were infested with termites, and on the 15th of February 2023 almost half of the pots were infested. The decision to move the plants from the greenhouse to the animal house was made, and on the 17th of February 2023 they received a treatment against termites (Undertaker 480 EC, Chlorpyrifos 480 g/L) that was sprayed outside of the pots as well as in the trays where the pots were placed. After this, no more termites were observed.

Some of the plants developed symptoms of some virus disease on the 20th of February 2023 (figure 4). It started of with some patchy chlorosis that later developed into necrosis of the older leaves and subsequently stunted and abnormal growth of the younger leaves as they emerged. Roughly one third of the plants were affected at the end of the experiment.

Figure 4: The symptoms on a plant in pot 28. Photo: Kari Løe

On the 27th of February 2023, 28th of February 2023 and 1st of March 2023 the plants were inoculated with four adult aphids per plant. Two weeks later, the 14th of March 2023 the aphids on each plant were counted. Pesticidal foliar treatments were applied directly afterwards. Each of the treatments were applied by spraying one litre of substance per 15 plants with the corresponding treatment. One week later, the 20th of March 2023, the aphids (both adults and nymphs) were counted again as well as the number of leaves. Plants were thereafter removed from the cocopeat and were measured from the tip of the main root to the top of the longest leaf. Subsequently, shoot tissues were placed in paper bags and placed into an oven to dry. The plants were weighed and then dried again until there was no fluctuation in weight so that the true dry matter weight was obtained. Throughout the experiment as some leaves died and fell off – these were collected and dried to get an accumulated dry weight at the end of the experiment.

2.3 Data analysis

The collected data was analysed in R (R Core Team 2023). Data wrangling and graphics used the package tidyverse in R (Wickham et. al 2019). To evaluate the effectiveness of rabbit urine on the productivity of kale and the pest density, linear models were created by inserting all possible predictor terms (nutrient, pesticide number of aphids) as well as possible interactions between them (nutrient:pesticide, pesticide:number of aphids). Partial F-tests determined if interaction terms did not contribute to model fit and thus could be removed, all main effects in all models were retained regardless of significance.

For the aphid response a generalized linear mixed effects model with poisson family and log link was created (Bates et. al 2015). The reason for using a generalized linear model was because the aphid response variable were counted quantitative data. Moreover, each plant started with a different number of aphids which entailed that the observations were not independent from each other (hierarchal). Some of the variance could be explained by fitting individual slopes to each plant (figure 5) which means that the mixed effects model was the right choice. For the mixed model, parametric bootstrap simulations (Halekoh, 2014) were performed to determine if higher order terms contributed significantly to model fit and to see if removing the term would lead to more unexplained variation than could be explained by chance. Only interactions that contributed to model fit were retained.

For the leaf growth response (number of leaves produced after treatment) a generalized linear model with quasipoisson family was used. The leaf growth response was also a count which means that a generalized linear model was prefered over a linear model. The quasipoisson family was used because the count variable was underdispersed (less variation than expected for the poisson family). $\mathbf{X^2}$ (Chi-squared) likelihood ratio tests were used to determine which terms contributed to model fit and which terms could be removed.

3. Results

3.1 Chemical properties of rabbit urine

The nitrogen content of the rabbit urine was 1.3%. The specific gravity was 1.0133 g/ml. 1.0133 g/ml * 100 ml * 0.013 = 1.317 g of nitrogen in 100 ml rabbit urine. Most of the nitrogen was in the form of urea (table 2). Other macro- and micronutrients were also present and pH and salinity were measured (table 2).

Table 2: Analysis results of rabbit urine.

Test	Results	Unit
Refractive index	1.33763	nD
pH	6.9	-
EC	25.05	microSiemen
Salinity, NaCl	2.75	%Mas
Specific Gravity	1.0133	Gram/ml
Total Dissolved solid, (TDS)	3.19	%Vol
Nitrogen	1.3	%Mas
Phosphorous	0.01	%Mas
Potassium	0.95	%Mas
Calcium	0.13	%Mas
Magnesium	0.55	%Mas
Osmotic Pressure	580.18	mOSm/L, (milliosmols)
% Brix	3.5	%Mas
% Glucose	3.28	%Mas
% Invert Sugar	3.31	%Mas
Sulfur	0.7	%Mas
Urea	1.41	%Vol.

3.2 Aphids

Table 3 shows the summary of the model used to test the change in aphids density over time (before-after). The model used was a generalized linear mixed effects model with poisson family (as the response variable is a count) and log link. The Rapid insecticide treatment had a negative effect on the aphids while the water and urine treatments showed an increase in the number of aphids (table 3). The nutrient treatment showed no evidence of having an effect on the pest density.

Terms with interactions between nutrient:pesticide and nutrient:time were not included as the Parametric Bootstrap test showed no evidence that these interactions had any effect on the number of aphids. The time variable refers to the time before and after the pesticidal treatment. However, the Parametric Bootstrap test showed moderate evidence (p = 0.0115) that the effect of pesticide depended on time interaction had effect on the number of aphids.

Table 3: The summary table of the model used for predicting the effect on aphids.). The '-' means that no p-value can be extracted, as one cannot take away a main effect that is included in a significant interaction term ina Parametric bootstrap test. In generalized models, the residual variance is fixed at 1. Significant p-values in bold. (Formula for the model used: Aphids ~ Nutrient + Pesticide + Time + (Time | Pot) + Pesticide:Time. The reference level set was Nutrient = water and Pesticide = water.)

effect	**group**	**term**	**estimate**	**variance**	**PB p-value**
fixed	NA	(Intercept)	3.56	0.138	**p < 0,001**
fixed	NA	NutrientUrine	0.168	0.181	0.30
fixed	NA	NutrientCAN	0.775	0.185	
fixed	NA	PesticideUrine	-0.428	0.18	-
fixed	NA	PesticideRapid	0.518	0.163	
fixed	NA	Timeafter	0.579	0.042	-
fixed	NA	PesticideUrine:Timeafter	-0.018	0.093	**0.0115**
fixed	NA	PesticideRapid:Timeafter	-8.246	1.115	
ran_pars	Pot	sd__(Intercept)	1.051	NA	NA
ran_pars	Pot	cor__(Intercept).Timeafter	-0.025	NA	NA
ran_pars	Pot	sd__Timeafter	0.726	NA	NA

The number of aphids increased after the application of the rabbit urine treatment, in the three nutrient treatment (figure 5). After treatment with the synthetic insecticide Rapid (for all nutrient treatments) all individual slopes as well as the predicted slopes show a strong decline in the number of aphids (figure 5). indicating no evidence that urine used as a pesticide has an effect against aphids.

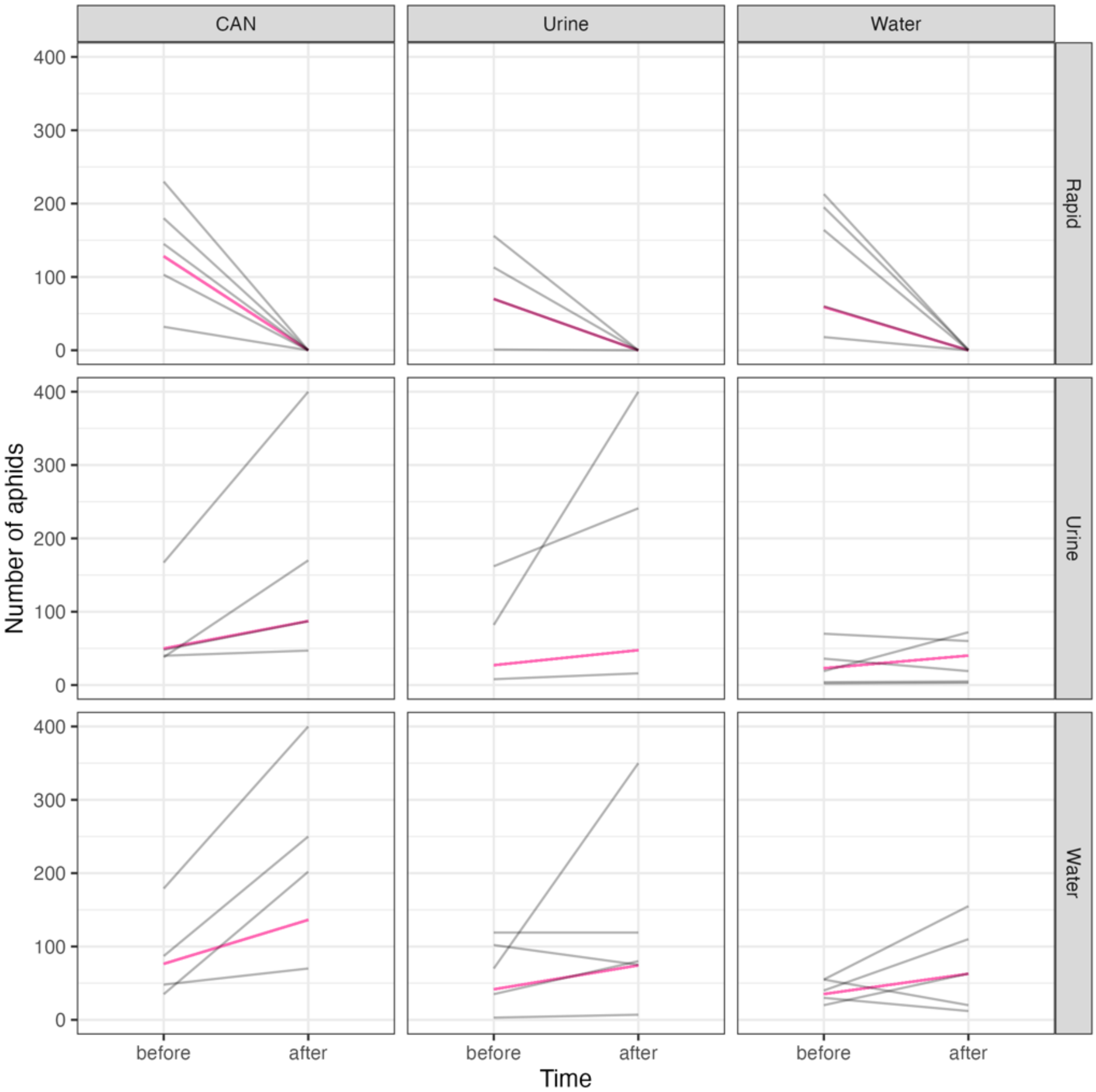

Figure 5: Change in aphids per treatment. The grey lines represent the individual plant and the pink lines represent the predicted changes for each treatment that the model provided. The columns represent the nutrient treatments and the rows represent the pesticide treatments.

3.3 Dry weight

The the nutrient treatments showed moderat evidence of having an effect on the productivity of the crops (p = 0.0187), which resulted in a higher dry weight of the kale after application of CAN and rabbit urine. The aphid count after the treatment also show weak evidence (p = 0.0443) of affecting the dry weight. The pesticide treatment showed no evidence (p = 0.2323) of affecting the dry weight (table 4).

Table 4: Summary table of the model used for predicting the dry weight. The reference level was set to CAN + Rapid (nutrient + pesticide) when aphids = 0. Significant p-values in bold. Model formula:Dry_weight ~ Nutrient + Pesticide + Aphids_after.

term	**estimate**	**variance**	**p.value**
(Intercept)	5.961	0.329	**p <0.001**
NutrientUrine	0.233	0.436	**0.0187**
NutrientWater	-1.611	0.453	
PesticideUrine	1.199	0.542	0.2323
PesticideWater	0.358	0.526	
Aphids_after	-0.006	0.00001	**0.0443**

Figure 6 shows the observations and predictions of the dry weight after the treatments. According to the prediction points it looks like CAN and urine should have a higher dry weight than that of the water treatment. However, the 95% confidence intervals overlap the means for CAN and Urine, but not for water.

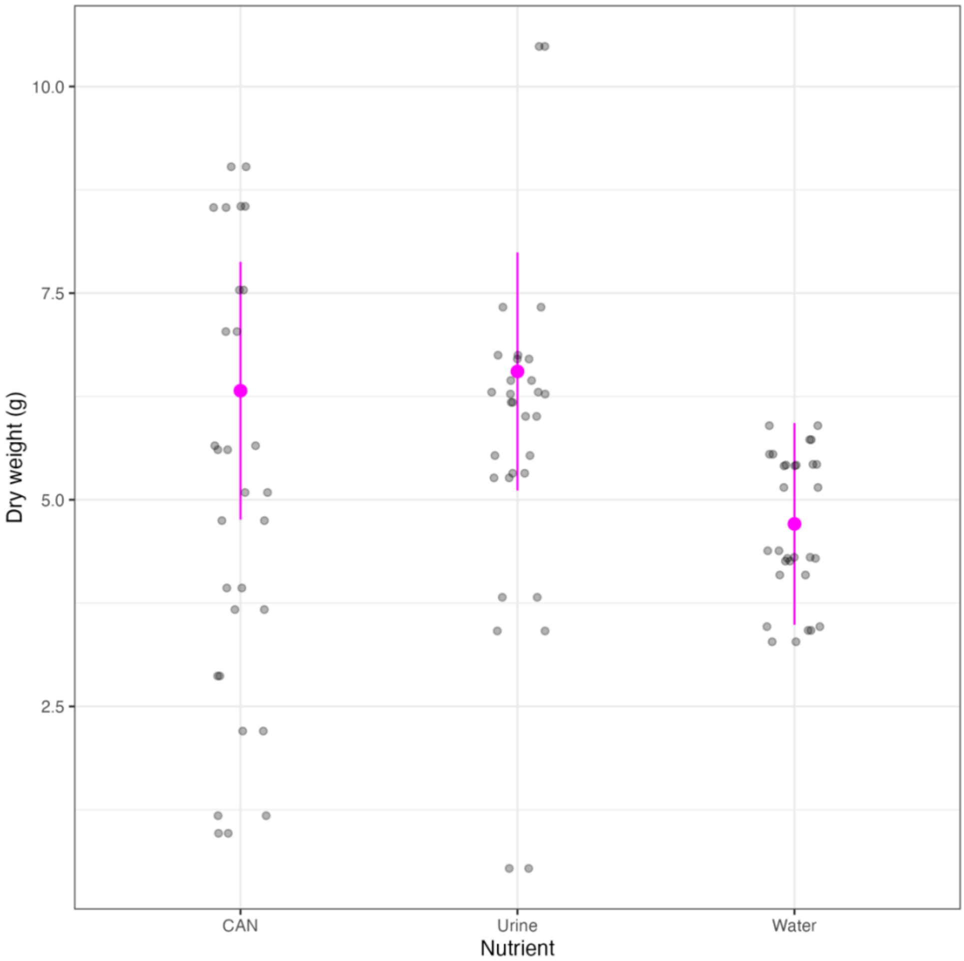

Figure 6: Observations and predictions of dry weight [g] per nutrient treatment. The grey dots represent each observation, the pink dot represents the the prediction of dry weight that the model provided when the number of aphids = 0 and the pesticide = Rapid. The pink lines represent the 95% confidence interval around the prediction.

3.4 Leaf growth

The nutrient treatment has strong evidence ($p < 0.001$) for affecting the number of leaves (table 5). The prediction of CAN seems to be higher than both the urine as well as the water treatments. The prediction for urine is also slightly higher than the water, however, the 95% confidence intervals for urine and water overlap each others means (figure 7). The pesticide treatment show weak evidence ($p = 0.031$) of affecting the amount of leaves produced (table 5). The prediction of Rapid seems to be higher than both the urine and water treatments. The prediction for urine is also slightly higher than the water, however, the 95% confidence intervals overlap the means for Rapid & urine as well as urine & water, but not fro Rapid & water (figure 8). There is no evidence ($p = 0.106$) that the number of aphids after the pesticidal treatment had any effect on the number of leaves produced (table 5). The prediction line increases slightly with aphid count. The observations are very scattered and the confidence interval is quite large (figure 9).

Table 5: The summary table of the model used for predicting the leaf growth. The Intercept is Nutrient CAN. The residual variance for generalized models is fixed at 1. Significant p-values in bold. (Model formula: Leaf_growth ~ Nutrient + Pesticide + Aphids_after)

term	**estimate**	**variance**	**p.value**
term	estimate	variance	p.value
(Intercept)	2.247	0.002	**p < 0.001**
NutrientUrine	-0.148	0.003	**p < 0.001**
NutrientWater	-0.217	0.003	
PesticideUrine	-0.098	0.004	**0.031**
PesticideWater	-0.172	0.004	
Aphids_after	3.922	5.570	0.106

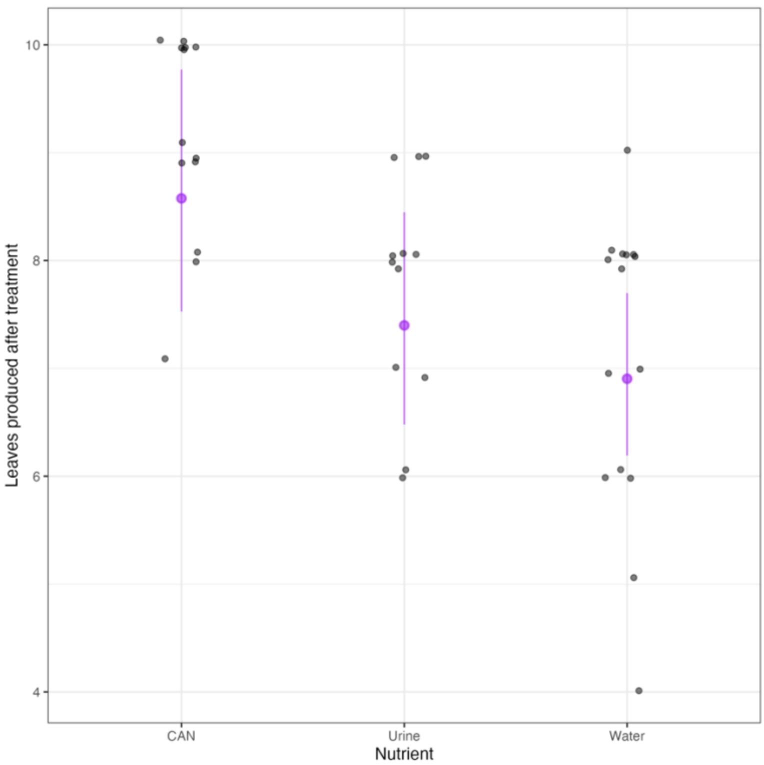

Figure 7: The partial effect of the different nutrient treatments on the number of produced leaves after the treatment started when the rererence is pesticide = urine and aphids =0 . The grey dots represent the observations, the purple dots represent the prediction provided by the model provided and the purple lines represents the 95% confidence interval.

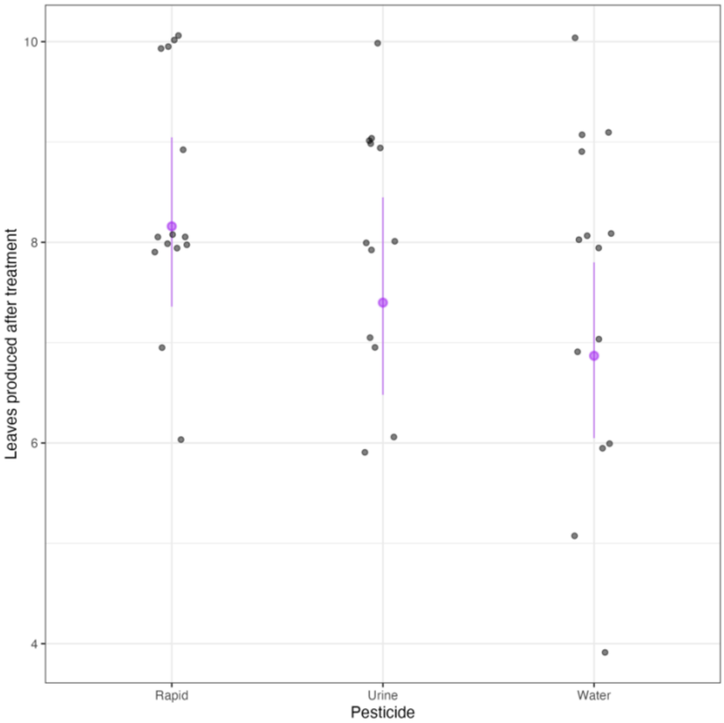

Figure 8: The partial effect of the different pesticide treatments on the number of produced leaves after the treatment started when nutrient = urine and aphids = 0. The grey dots represent the observations, the purple dots represent the prediction provided by the model provided and the purple lines represents the 95% confidence interval.

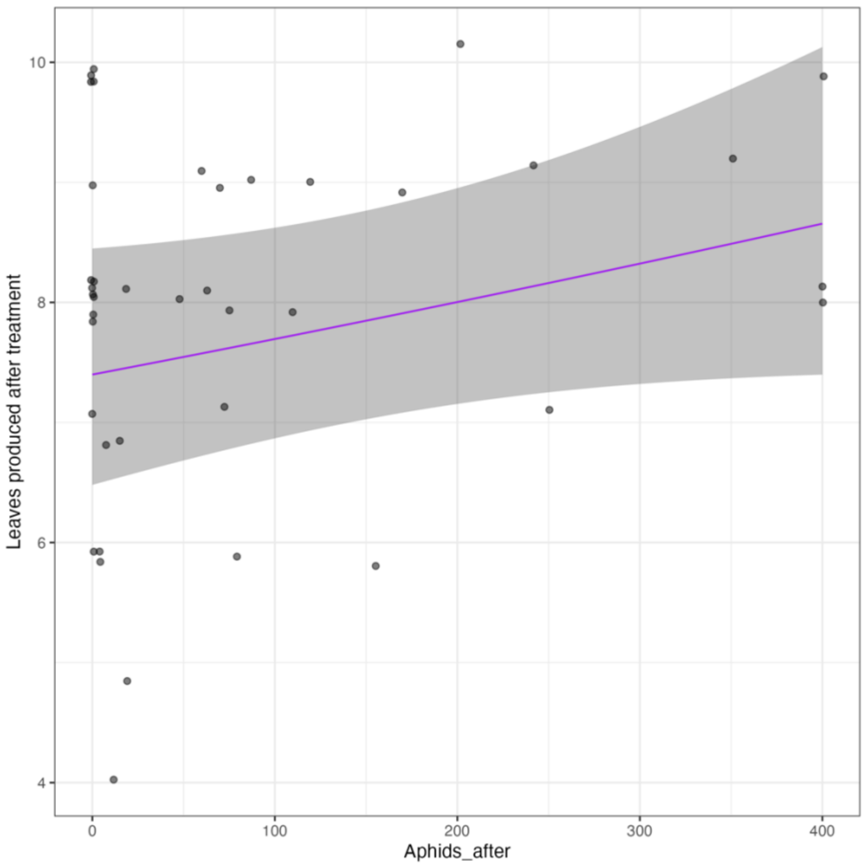

Figure 9: The partial effect of the number of aphids (after treatment) on the number of produced leaves when nutrient = urine and pesticide = urine. The grey dots represent the observations, the purple line represents the prediction provided by the model provided and grey ribbon represents the 95% confidence interval.

4. Discussion

The purpose of this study was to evaluate how effective rabbit urine is to increase crop productivity and compare this to a synthetic commercial fertilizer, as well as to evaluate the efficacy of rabbit urine on decreasing aphid population density compared to a commercial insecticide.

4.1 Discussion of the methods

The urine analysis was only carried out on the first batch of rabbit urine, and contents could have differed slightly in the second batch due to possible changes in the rabbit's diets. For example, if the rabbits suddenly got more protein in their diet the later batches could potentially have had a higher nitrogen content than the batch that was analyzed. However, both of the batches of rabbit urine were procured from the same farm. There is no information on the diets of the rabbits. To get 2 litres of urine, the urine of four rabbits were collected over the course of one week.

The substrate used for planting the Sukuma wiki plants was cocopeat – a nutrient deficient substrate made out of coconut fibres. Because of its lack of nutrients 20 g of DAP fertilizer had to be mixed into the pots before planting to avoid that the seedlings would die from malnutrition before the experiment started. However, it turned out that the amount of nitrogen applied in the beginning was 3,6 g per pot and the amount of nitrogen applied for the experiment was approximately 1,3 g for the urine and CAN respectively. The starter amount of nitrogen was relatively high compared to the additional nitrogen, so perhaps the fertilizer effect would have been larger if the amount of nitrogen applied at the start had been smaller, or perhaps the treatments more intense.

Another problem was that when the termites infested the pots, they created tunnels and ate a large amount of the cocopeat fibres. This could potentially have contributed to leaching/washing out of nutrients as the liquid would drain out of the holes created by the termites. This proved to be a problem when applying the urine as some leached out of the pots even though it was poured slowly into the pots to try and avoid overflow and to give it time to absorb. This was not a problem for the CAN as it's granular form allowed it to be released more slowly into the soil with each watering. An improvement of the method could be to divide the total 200 ml of 50 % diluted rabbit urine into several applications to make sure that all moisture got absorbed into the substrate or perhas dry the urine into pellets to make the nutrients release slower into the soil. However, since some of the urine leached out of the pots and the dry weight prediction for urine still was higher than for CAN, it indicates that the urine nutrient treatment could be more effective than the CAN treatment.

When drying the plants in the ovens there was a problem with condensation. One of the ovens was faulty and did not ransport the excess moisture away properly so 3 of the plants were completely soaked in water and had started to mold and rot. The bags were changed and dried in another oven, but it was difficult to get a good measurement of the dry weight as some of the plant matter was stuck to the paper bags which may have impacted the results slightly.

4.2 Discussion of results

4.2.1 Pesticidal effects

The results show that the the commercial pesticide (Rapid) is a very effective at controlling aphid population growth (figure 5). However, there was no evidence that 50% diluted rabbit urine as a control agent against aphids had any effect different from water. In previous studies where evidence for pesticidal effects have been found the concentration of livestock urine has been higher (75-100%), and only 50% dilution was used in this experiment, which could explain the lack of effect. The study made by Kemunto et. al (2022) was performed with fall army worms and showed a higher mortality due to reduced feeding on the plant material. In this experiment aphids were used, which give birth to live nymphs that feed on the sap. Perhaps this is a key difference to why pest mortality did not increase – the juveniles of aphids feed directly on the sap and not the plant tissue and therefore the preference for feeding was the same as the sap did not change taste. Perhaps if the experiment went on longer sublethal effects such as lifespan, fertility or oviposition (in colder climates where males are produced) could be observed.

4.2.2 Nutritional effect

For the plant productivity (dry weight) the results show that there is evidence to suggest that the rabbit urine increases the productivity of the kale plants in comparison to water, and the prediction from the model is even slightly higher than that of the CAN fertilizer.

For the leaf growth effect there are three partial effects to be discussed. Firstly, the nutritional effect seemed to be highest for CAN, slightly lower for urine and lowest for water. This suggests that rabbit urine helps the plant produce more leaves than water, but not as much as CAN. The pesticide treatment also seems to have an effect on the leaf growth. The Rapid pesticide treatment showed slightly higher leaf growth than that of the urine and water treatments. This could be explained by the fact that aphids cause stunted growth and the Rapid killed all aphids and perhaps then helped the plant produce more leaves. The number of leaves also increased slightly with number of aphids. However, the number of observations for the higher aphid numbers are very few. If the sample size was larger so the number of observations in higher aphid number were more – perhaps the prediction line would look different The increase is also very small- according to the prediction the number of leaves for 0 aphids would be approximately 7,5 and for 400 aphid the number of leaves would be 8,7, so this effect is marginal and measured with considerable uncertainty. Nonetheless, it could be possible that more leaves makes it possible for more aphids to establish and build healthy populations on the plant. A study by Hosseini et. al (2009) found that the number of offspring in cotton aphids *Aphis gossypii* increased on cucumber plants that had higher nitrogen fertilization than plants with lower leels of nitrogen fertilization. The reason for this being that nitrogen is essential and a limiting factor for growth in insects (Hosseini et. al 2009). Higher nitrogen content could then mean more leaves, but the high nitrogen content in the leaf tissue alo contribute to aphid growth and increased birth rate (more aphids).

One important thing to note is that two species (*Myzus persicae* and *Brevicoryne brassicae*) were used for the project, as there were not enough aphids for all plants to be inoculated with only one species. It was not taken into account that these two species might have different growth rates, generation times, competition between the species or other key attributes that potentially could have impacted the results of the study. For future studies it would be beneficial to only use one species to eliminate such variation.

4.2.3 Conclusions

As has been demonstrated by the research in this experiment, rabbit urine is a viable option to use as a low cost biofertilizer. To the contrary, there is no evidence from this experiment to suggest that rabbit urine has any effect on aphids as a biopesticide.

The recommended spacing for Mfalme F1 variety of Sukuma wiki is 60 cm x 60 cm (Greenlife Crop Protection Africa 2023). This gives 11241 plants per acre. The amount of urine needed if 100 ml is needed for one plant is 1124100 ml or 1124 litres per acre. One rabbit produced approximately 0.5 liters per week, which means that 2248 rabbits are needed to produce the amount necessary for one acre of Sukuma wiki in one week. However, small scale farmers contribute 78% of Kenyas agricultural output and these farmers have a farm size averaging at 0.2-3 ha (World bank 2015). If these farmers grown Sukuma wiki on 0.1 ha (0.247 acres) of their land (probably even less), 278 litres of rabbit urine is needed, which is the urine from 555 rabbits in one week or 46 rabbits in 3 months. This is quite a large amount of rabbits and is perhaps not attainable for most small scale farmers. However, even a small number of rabbits on a farm could be a valuable supplement to commercial synthetic fertilizers.

In short, rabbit urine is areadily available source of nitrogen that smallhold farmers with rabbits can utilize to increase plant productivity at a rate comparable to synthetic commercial fertilizers that are more have larger environmental impact. However, the amount needed is large and the urine may be best suited as a complementary source of nitrogen for small scale farmers and not as the primary source, unless the farm have a large number of rabbits available.

4.3 Future studies

To further investigate the pesticidal properties of rabbit urine it could be interesting to explore different concentrations of urine for foliar application. To avoid scorching of the leaves the urine could be fermented and thus be used in higher concentrations. It could also be interesting to evaluate if spraying on several occasions could decrease the pest population numbers more than just the one occasion that was used in this experiment. Furthermore, it could also be interesting to evaluate the effects of rabbit urine in combinations with other bio pesticides such as neem oil as was done with cow urine by Singh et. al (2019).

Future studies on the effect of rabbit urine as a fertilizer should be done in field trials on different crops to measure yield. With field trials the substrate problem is avoided, as the experiment is performed in natural conditions.

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