BANANA FIBRE PAPER AS A SUSTAINABLE NEMATICIDE DELIVERY SYSTEM FOR IMPROVED NEMATODE MANAGEMENT, CROP PRODUCTIVITY AND SOIL HEALTH

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A thesis submitted in partial fulfillment for the requirements of the award of the degree of Doctor of Philosophy in Crop Protection in Masinde Muliro University of Science and Technology

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DECLARATION

This thesis is my original work prepared with no other than the indicated sources and support and has not been presented elsewhere for a degree or any other award.

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance of Masinde Muliro University of Science and Technology a thesis entitled "**Banana Fibre Paper as a Sustainable Nematicide Delivery System for Improved Nematode Management, Crop Productivity and Soil Health**".

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DEDICATION

This work is dedicated to my beloved husband Joel Ogembo and in loving memory of our dad Christopher Ogembo. I dedicate also to my dearest brothers Dr. Patrick Atandi and Dr. David Atandi.

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ABSTRACT

Potato (*Solanum tuberosum*) and green pea (*Pisum sativum*) are important staple and legume crops in Kenya, yet their productivity is increasingly constrained by plant parasitic nematodes (PPN), including root-knot nematodes (*Meloidogyne* spp.) and potato cyst nematodes (*Globodera* spp.), as well as other biotic and abiotic stresses. Traditional nematode management approaches, including chemical nematicides, face limitations such as environmental toxicity, high cost, and restricted applicability, highlighting the need for integrated, sustainable alternatives. This study evaluated the efficacy of banana fibre paper technology, biodegradable paper treated with abamectin, fluopyram, or *Trichoderma asperellum,* on PPN suppression, potato cyst nematode reproduction, crop yield, and soil health in field trials in Nyandarua and Nyamira counties. Four field experiments were conducted across multiple cropping seasons, including treatments of abamectin-paper, fluopyram-paper, *Trichoderma*-paper, untreated paper, and controls. Nematode populations were monitored at planting and harvest while potato cyst nematode reproduction, crop yield, and soil health indicators, including free-living nematode (FLN) abundance, ecological indices, and microbial biomass were assessed at crop harvest. Data were analysed using ANOVA with significance set at $p \leq 0.05$. Results revealed that banana fibre paper treatments significantly reduced PPN densities: abamectin-paper reduced nematodes by up to 48%, while fluopyram-paper achieved 58–72% reductions depending on season and cultivar. *Trichoderma*-paper resulted in 40–65% suppression and consistently increased yields. In peas, suppression was minimal and did not translate into yield gains. *Trichoderma*-paper enhanced FLN abundance and diversity, increased enrichment and structure indices by 25–40%, and boosted microbial biomass by up to 60%, whereas chemically treated papers, particularly fluopyram, elevated basal indices and lowered fungal-to-bacterial ratios, suggesting reduced soil food web complexity. This study provides novel evidence that integrating chemical and biological nematode control within a slow-release biodegradable matrix can suppress PPN while conserving or enhancing soil ecological function under smallholder conditions. The findings offer a practical, and cost-effective nematode management approach for farmers in Nyandarua and Nyamira. It is recommended that future adoption focus on *Trichoderma*-enriched banana fibre paper and optimize pelletized formulations for improved field efficiency and ease of use.

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ABBREVIATIONS AND ACRONYMS

AEZ	Agro-Ecological Zone
AMF	Arbuscular Mycorrhizal Fungi
ANOVA	Analysis of Variance
BI	Basal Index
CAN	Calcium Ammonium Nitrate
CGN	County Government of Nyamira
CI	Channel Index
CIP	International Potato Center
CoG	Council of Governors
c-p	Colonizer-persister
cv	Cultivar
DAP	Di-ammonium Phosphate
EFSA	European Food Safety Authority
EI	Enrichment Index
EPN	Entomopathogenic Nematodes
EPPO	European and Mediterranean Plant Protection Organization
F:B	Fungal-to-Bacterial
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization of the United States
FLN	Free-Living Nematodes
GLM	Generalized Linear Model
GoSA	Government of South Africa
HSD	Honest Significant Difference
icipe	International Center for Insect Physiology and Ecology

IITA	International Institute of Tropical Agriculture
IPM	Integrated Pest Management
KALRO	Kenya Agricultural and Livestock Research Organization
KSU	Kansa State University
LM	Lower Midland
Ln	Natural Log
LSD	Least Significant Difference
MI	Maturity Index
MMUST	Masinde Muliro University of Science and Technology
MoAFL	Ministry of Agriculture, Fisheries and Livestock
N_2	Dinitrogen
NC	North Carolina
NCSU	North Carolina State University
NH_3	Ammonia
NH_4^+	Ammonium ion
NINJA	Nematode Indicator Joint Analysis
NPCK	National Potato Council of Kenya
NY	New York
PCN	Potato Cyst Nematode
PCNBW	Potato Cyst Nematode Bacterial Wilt
Pf	Population final
Pi	Population initial
PPN	Plant Parasitic Nematodes
RCBD	Randomized Complete Block Design
RF	Reproduction Factor

RKN	Root Knot Nematode
RKNBW	Root Knot Nematode-Bacterial Wilt
SDH	Succinate Dehydrogenase
SDHI	Succinate Dehydrogenase Inhibitors
SI	Structure Index
TSP	Triple Super Phosphate
UH	Upper Highland
UK	United Kingdom
UM	Upper Midland
UNEP	United Nation Environmental Program
UNESCO	United Nations Educational, Scientific and Cultural Organization
USA	United States of America
USD	United States Dollar
USDA	United States Department of Agriculture

CHAPTER ONE: INTRODUCTION

1.1 Background

Agricultural production is the backbone of the Kenyan economy whereby Kenya grows a variety of crops for both export and domestic use (Nzomoi *et al.*, 2022). Worldwide, potato (*Solanum tuberosum*) ranks as the fourth most important staple food after maize (*Zea mays*), rice (*Oryza sativa*) and wheat (*Triticum aestivum*) (Lima *et al.*, 2018; Sanwal *et al.*, 2022). Potato production has been on the rise in Kenya currently ranking at second position after maize (CIP, 2021; Ahmadu *et al.*, 2021). They are grown in most parts of Kenya but mostly in Nyandarua and Elgeyo Marakwet counties (Kaguongo *et al.*, 2014). On the other hand, peas (*Pisum sativum*) are considered amongst the most important vegetable and commercial crop in Kenya (Umar, 2024). Some cultivars like snow peas and garden peas are grown for export bringing returns of KES 1 billion+ in 2005 (Lengai *et al.*, 2022). Similar to other legumes, peas contain the bacteria *Rhizobia* in the root nodules and are therefore capable of fixing nitrogen from atmospheric nitrogen (N_2) to ammonia (NH_3) then into ammonium (NH_4^+) which is plant-usable thus improving soil fertility (Dhall, 2017; Wu *et al*, 2018; Mekonnen *et al.*, 2023; Chamoli *et al.*, 2024; Kumar *et al.*, 2025). Like most agricultural crops, the productivity of potatoes and peas is negatively affected by both biotic and abiotic factors with nematodes being a key biotic challenge (Mburu *et al.*, 2020).

Plant parasitic nematodes (PPN) have been documented as a serious problem in almost all crops in agroecosystems (Perry and Moens, 2013; Perry *et al.*, 2024). Depending on the crop and host, they may reduce crop production by up to 100% (for example *Globodera* spp. in potatoes), although the average range is between 11-20% annually worldwide (Abd-Elgawad, 2014; Ansari *et al.*, 2020). They are found mostly in the rhizosphere and live in the interconnected soil pores formed by soil processes (Yeates and Newton, 2009; Kumar *et al.*, 2019). They are known to cause yield losses by attacking crops either directly or indirectly.

The most common attack is direct whereby they feed on the host roots using a stylet to drain nutrients (Coyne *et al.*, 2014; Heuer *et al.*, 2021). Indirectly, some PPN act as vectors, so they transmit plant viruses such as tomato leafspot virus (Ravichandra, 2013; Parrado and Quintanilla, 2024). Furthermore, their feeding usually creates openings for other plant pathogens such as bacteria and fungi to enter (Bhat *et al.*, 2023) and sometimes they form disease complexes which lead to even greater yield losses (Singh *et al.*, 2022).

Potato cyst nematodes (*Globodera* spp.) and root knot nematodes (*Meloidogyne* spp.) are arguably the most damaging nematodes to potatoes and most crops worldwide, respectively (Lima *et al.*, 2018; Bali *et al.*, 2021). They are sedentary nematodes meaning they establish a feeding site inside the host root tissue and seize to be mobile. Potato cyst nematodes (PCN) are highly host-specific pathogens which feed strictly on potatoes, but also on plants belonging to Solanaceae family in the absence of potatoes (Mburu *et al.*, 2018; Ochola *et al.*, 2020). They are considered as the most threatening nematodes to potatoes globally causing heavy yield losses of up to 12% estimated at USD 173 billion annually (Coyne and Affokpon, 2018; Gamalero and Glick, 2020; Mburu *et al.*, 2020; Lawaju *et al.*, 2021). Contrarily, root knot nematodes (RKN) are polyphagous feeding on a wide range of crops causing significant losses to crop yield and quality (Akinsanya *et al.*, 2020; Khan *et al.*, 2023a; Kolombia and Fabiyi, 2023). They tend to form galls on the roots and tubers of host plants which reduces their ability to absorb water and nutrients from the soil and also making them unmarketable (Forghani and Hajihassani, 2020). In addition, they are cosmopolitan having a worldwide distribution in both tropical and temperate environments.

Management of nematodes has often been based on use of chemical nematicides (Faske and Starr, 2006; Faske and Hurd, 2015; Oka, 2020; Phani *et al.*, 2021; Jones *et al.*, 2022), biological control using microorganisms (Abd-Elgawad, 2020; Kiriga *et al.*, 2018), physical control like solarization and flooding (Kaskavalci, 2007; Efthimiadou *et al.*, 2009; Jamshidnejad *et al.*, 2013; Ntinokas *et al.*, 2025), organic control using organic amendments (Atandi *et al.*, 2017; Mandal and Hossain, 2017; Singh *et al.*, 2018), among others. However, it is becoming more apparent that the most sustainable approach to nematode control will integrate several strategies and techniques, including cover crops, crop rotation, organic soil amendments, least-toxic pesticides, and planting of resistant cultivars (Atandi, 2018).

Banana fibre paper technology is a climate-smart innovation that integrates physical, chemical or biological strategies in the control of PPN (Pirzada *et al.*, 2018). It involves the use of a biodegradable lignocellulose matrix as a carrier for low dose nematicides or microbes. Prior to selection, paper selection studies were conducted, and they revealed banana paper to be the most suitable material as an organic carrier (Pirzada *et al.*, 2020). Properties of the paper enable the application of chemical nematicides in extremely low doses, or biologically based products, such as beneficial fungal antagonists. It has been successfully used in the control of PPN on several crops across East and West Africa (Affokpon *et al.*, 2018; Cao *et al.*, 2022; Dedehouanou *et al*., 2022; Ochola *et al.*, 2022). This technology presents a promising option towards addressing the nematode challenge. Therefore, this study aims to investigate the potential of the banana fibre paper as an organic carrier in potato and pea cropping systems.

1.2 Statement of the problem

The population of Kenya is at a rapid increase according to the 2019 census and yet due to urbanization, the size of arable land is declining. This consequently increases the demand for food to ensure food security and therefore, there is a need to maximize crop production through pest and disease control. Following the detection of the two most important potato cyst nematodes (PCN) species in Kenya, *Globodera rostonchiensis* and *G. pallida*, as well as declining yields per hectare, it has become necessary to employ stringent management measures to curb the nematode pest problem urgently. A set of innovations that could result in increased potato production such as use of fertilizers, clean seeds, resistant cultivars and chemicals have been suggested, explored and some have even been adopted (Breisinger *et al*., 2023).

Nematicides have often been considered as the most effective strategy in nematode management, however, the decreased availability and acceptability of nematicides in the past (Abd-Elgawad, 2024) has left a gap in how to effectively manage nematode populations. Despite numerous studies showing the high potential of soil organisms as biocontrol agents, farmers lack the high technical skills required for their application. This has created a need to establish suitable integrated management methods such as the banana fibre paper technology.

Banana fibre paper technology has already shown promise as a biodegradable delivery system for nematicides in several tuberous crops, including yams and potatoes (Dedehouanou *et al*., 2022; Kamau *et al*., 2024). However, its application remains confined to abamectin delivery, with no investigations assessing its compatibility with other effective nematicides such as fluopyram. Moreover, limited research has explored its comparative effectiveness across potato cultivars with differing resistance levels. Although the technology has been tested in solanaceous crops, its potential role in nematode management for legumes, particularly green peas, remains largely unexamined, despite growing reports of nematode-related damage in these systems.

Finally, there is a lack of empirical data on the effect of banana fibre paper treatments on non-target soil organisms which are critical indicators of soil health and biological function. Soil microbes, including free-living nematodes and beneficial microbes, play key roles in nutrient cycling, plant growth promotion, and ecosystem stability. Free-living nematodes are increasingly recognized as sensitive indicators of soil ecological function due to their roles in carbon and nitrogen cycling and their widespread presence across soil habitats. In addition to PPN control, banana fibre paper as an organic amendment could prove beneficial as organic amendments are known to enhance soil biodiversity and health by stimulating beneficial microorganisms and soil fauna (Tuck *et al*., 2014; Lori *et al*., 2017; Anyango *et al*., 2020; Stein-Bachinger *et al*., 2021; Atandi *et al*., 2022).

1.3 Justification of the study

This study contributes important knowledge on sustainable approaches for managing plant parasitic nematodes in potato- and pea-based farming systems, particularly within smallholder contexts in sub-Saharan Africa. The use of biodegradable treated banana fibre paper as a low-cost, environmentally safe, and effective method for nematode control has the potential to support increased crop productivity while reducing the financial burden on farmers. By enabling growers to achieve better yields with fewer chemical inputs, the technology aligns with ongoing efforts to promote more accessible and ecological plant health solutions. In addition, the study helps to strengthen local capacity in applied nematology by generating findings that can inform practical, farmer-adoptable management options.

It also evaluates the broader effects of these treatments on non-target soil organisms such as free-living nematodes and beneficial soil microbes, offering insight into soil health dynamics under different nematode control strategies. This integrated view is essential for developing pest management systems that improve productivity while maintaining ecological balance. The findings are expected to guide future research and extension and support informed decision-making at both farmer and institutional levels, particularly in the context of reducing over-reliance on synthetic agrochemicals.

1.4 Significance of the study

This research provides critical insights into an emerging biodegradable delivery technology as a novel platform for deploying chemical and biological nematicides within potato- and pea-based smallholder systems in Kenya. As potatoes remain a strategic food security crop in East Africa and the fourth most important food crop globally (Lima *et al*., 2018), sustained productivity is vital for regional livelihoods and national nutrition goals. Peas, although widely cultivated in highland regions for income and dietary diversification, have received limited attention in nematode research despite their growing economic role.

By evaluating banana fibre paper enriched with fluopyram, abamectin, and *Trichoderma* spp. across multiple field seasons and agro-ecological zones, this study advances integrated nematode management innovations that align with climate-smart, resource-efficient agriculture. The findings strengthen scientific understanding of how biodegradable paper matrices influence plant parasitic nematode suppression, crop yield, and soil ecological balance. Furthermore, the research provides evidence on the potential of biocircular agricultural waste streams to enhance soil health indicators, including free-living nematode diversity and microbial biomass. Overall, this study contributes to the development of nature-based, farmer-feasible nematode management strategies, offering a scalable pathway toward reduced dependence on synthetic agrochemicals, enhanced soil functioning, and sustainable intensification in smallholder farming systems in sub-Saharan Africa.

1.5 Objectives

1.5.1 General objective

To evaluate the efficacy of chemically and biologically treated banana fibre paper in managing nematodes and enhancing crop performance and soil health in potato- and pea-based systems.

1.5.2 Specific objectives

The specific objectives were to:

i. Quantify and characterize plant parasitic nematode assemblages under chemically treated banana fibre paper in potato fields.
ii. Compare the effects of direct versus paper-incorporated nematicide application on nematode suppression and yield performance in potatoes.
iii. Evaluate the influence of chemically and biologically treated banana fibre paper on nematode populations in potato and pea cropping systems.
iv. Assess soil health responses to treated banana fibre paper using free-living nematodes and microbial indicators.

1.6 Hypotheses

i. $\mathbf{H_{01}}$**:** There is no difference in plant parasitic nematode assemblage composition or density between chemically treated banana fibre paper and control.
ii. $\mathbf{H_{02}}$: There is no difference in nematode suppression or yield between direct nematicide application and paper-incorporated nematicide application in potato.
iii. $\mathbf{H_{03}}$: Chemically and biologically treated banana fibre paper have no effect on plant-parasitic nematode population densities in potato and pea cropping systems.
iv. $\mathbf{H_{04}}$: Treated banana fibre paper does not affect soil biological indicators (free-living nematode community structure and microbial biomass).

1.7 Scope and limitations of the study

This study focused on evaluating chemically and biologically treated banana fibre paper as an innovative, biodegradable technology for managing plant-parasitic nematodes and enhancing soil health in potato- and pea-based cropping systems. Field experiments were conducted under smallholder production conditions in selected sites representing major potato-growing regions in Kenya. The research included chemical treatments (fluopyram- and abamectin-treated papers) and biological treatments (*Trichoderma*-treated paper), compared against untreated paper and unwrapped controls. It assessed nematode assemblages, population dynamics, and suppression levels, while comparing the efficacy of direct nematicide application versus paper-incorporated delivery methods. Soil health indicators, including free-living nematodes and microbial biomass, were measured to capture ecological impacts, and yield performance of potato and pea crops was recorded to establish agronomic relevance.

The scope was limited to the experimental sites, treatment combinations, and cropping cycles within the study period. Trials in Nyandarua and Nyamira counties may not capture the full soil and climatic variability of other regions. The banana fibre paper wrapping method, though effective at small scale, may pose logistical challenges for large-scale mechanized systems. While nematode indicators and microbial biomass were assessed, more detailed microbial community analyses (e.g., molecular sequencing) were beyond the study's scope. Long-term effects, including residual impacts in subsequent cropping seasons, were not evaluated. Broader socioeconomic and economic analyses of banana fibre paper adoption were also beyond the current study but are recommended for future research.

CHAPTER TWO: LITERATURE REVIEW

2.1 Crop production

2.1.1 Potato production in Kenya and disease constraints

Potato was introduced in Kenya in the 1880s by British colonialists and increased in popularity from the 1920s (Mwakidoshi *et al.*, 2021). Today, Kenya may be classified as a high potential potato-growing country with 161,000 hectares of potatoes grown annually (Borus and Parker, 2020). About 800,000 small holder farmers grow potatoes making it the second most important food crop in Kenya after maize (Velesi, 2018; Thuo and Maina, 2024). This has consequently generated employment and income for over 2.5 million people along the potato value chain (Abong' and Kabira, 2013). Potatoes are said to provide more food per hectare than other staple foods owing to their short maturity period of 85-120 days with at least two seasons per year (Mburu *et al.*, 2020). Small holder farmers often yield 6-7 t/ha whereas large scale farmers achieve higher yields of between 10-14 t/ha (Yildiz and Ozgen, 2021).

Irish potato production is affected by both temperature and rain (Ndegwa *et al.*, 2020). Favourable climatic conditions are in areas with 850-1200 mm of annual rainfall and altitudinal range of 1500-2800 m above sea level (Haro, 2022). These areas are concentrated in Central (Nyandarua county), Eastern (Meru and Embu counties) and Rift valley (Elgeyo Marakwet and Nakuru counties) regions of Kenya (Cheruiyot *et al.*, 2023; Vollmer *et al.*, 2022) (Figure 2.1). More counties are slowly venturing into potato farming including Taita Taveta, Pokot, Nandi, Uasin Gishu, Bungoma and Busia (Wanjira and Muriuki, 2020). A number of potato cultivars are currently grown in Kenya with varying preferences among farmers (Mugo *et al.*, 2021) (Table 2.1).

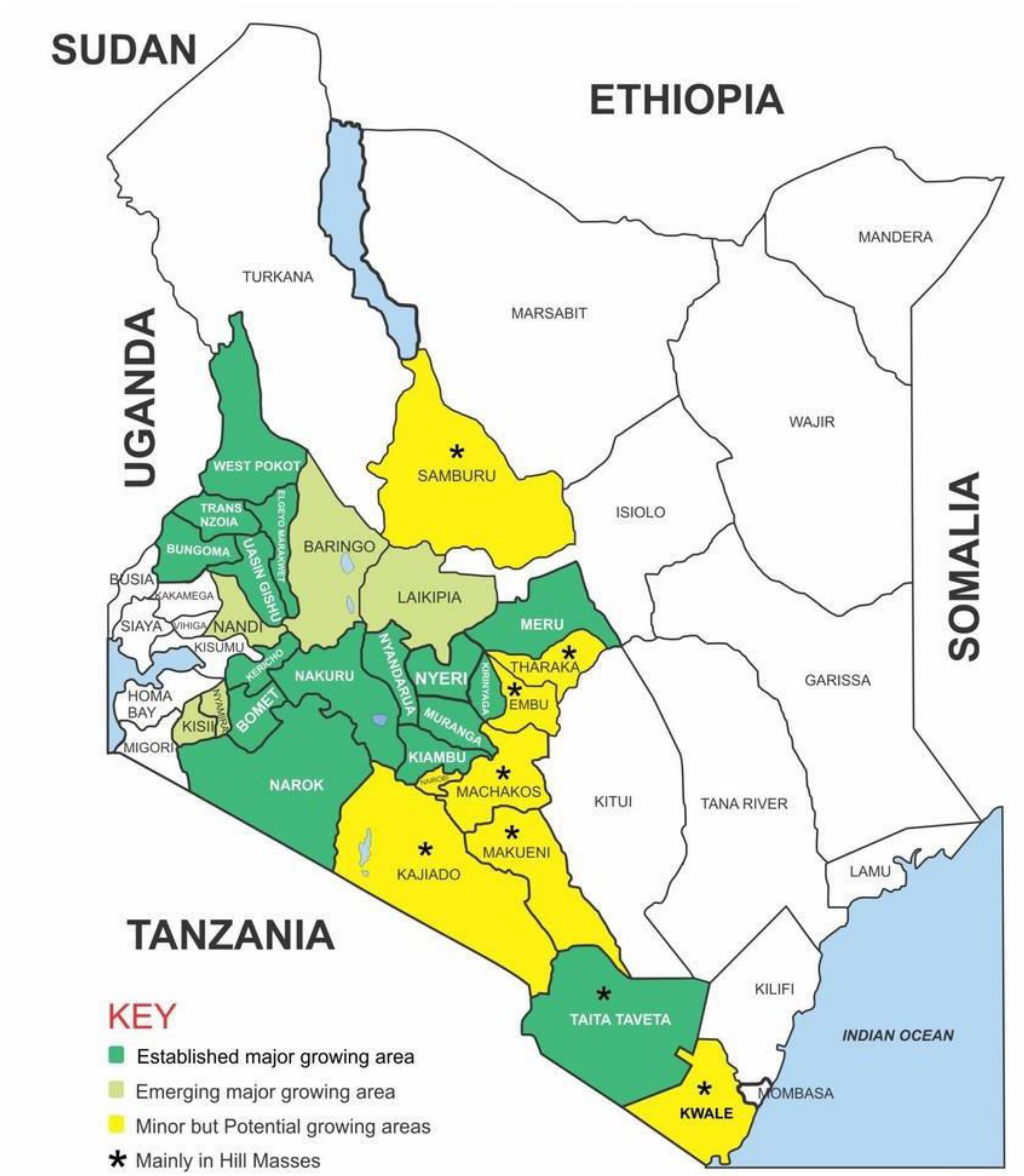

Figure 2.1 Map showing the major and minor potato growing areas in Kenya

Source: Mbugua *et al*. (2017)

Table 2.1 Main potato cultivars grown in Kenya and their attributes (adopted from NPCK, 2023).

Cultivar	Release year	Owner	Time to mature (days)	Yield (t/ha)	Attributes
Shangi	2015	KALRO	≤ 90	30 – 40	Chips, table variety, moderately susceptible to Late Blight, smooth cream skin
Dutch Robjin	1960	KALRO	90 – 120	≤ 30	Crisps, moderately resistant to Late Blight, red skin
Asante	1998	KALRO/CIP	90 – 120	35 – 45	Table variety, tolerant to Late Blight, smooth pink skin
Desiree	1972	KALRO	75 – 105	35 – 40	Chips, table variety, moderately tolerant to Potato Virus Y, smooth red skin
Sherekea	2010	KALRO/CIP	90 – 120	>40	Crisps, chips, table variety, resistant to Late Blight, PVY and PLRV, smooth red skin
Tigoni	1998	KALRO	90 – 120	35 – 40	Frozen chips, table variety, moderately tolerant to Late Blight, smooth white skin
Unica	2016	CIP	≤ 90	>40	Chips, crisps, table variety, moderately resistant to Late Blight, highly resistant to PVX and PLRV

Despite more land being devoted to potato production, Kenya's yields remain low averaging at 9-10 tonnes per hectare which is four times below the potential yield (Wang'ombe and Dijk, 2013; Kiptoo *et al.*, 2016; FAO, 2017; VIB, 2022; Ochieng, 2023). This may be attributed to several biotic and abiotic factors including poor seed quality or use of uncertified seed tubers, climate change, major disease outbreaks, wrong cropping practices by farmers due to lack of knowledge and capacity, poor post-harvest handling of produce, and lack of market plus fluctuating market prices, among others (Kaguongo *et al.*, 2014; Dijkxhoorn *et al.*, 2019). Of the common pest and diseases, the major constraints appear to be late blight (caused by *Phytophthora infestans*) and bacterial wilt (caused by *Ralstonia solanacearum*) as the main fungal and bacterial diseases, respectively (CIP, 2021). In addition, viral diseases such as potato virus X, potato virus Y, potato virus A, potato virus M, and potato leaf roll virus (Aleem *et al.*, 2018; Polder *et al.*, 2019; Onditi *et al.*, 2021) as well as plant parasitic nematodes (PPN) (Coyne *et al.*, 2018a; Niere and Karuri, 2018; Mburu *et al.*, 2020) are now becoming serious constraints.

The potato cyst nematodes (*Globodera rostochiensis* and G. *pallida*) which are quarantine pests that were recently detected in Kenya (Mwangi *et al.*, 2015; Mburu *et al.*, 2018) along with the root knot nematodes (*Meloidogyne* spp.) are considered the most damaging species of potatoes (Coyne and Affokpon, 2018; Coyne *et al.*, 2020). Other important nematodes of potatoes include the false root-knot nematode (*Naccobus* spp.), lesion nematode (*Pratylenchus* spp.), potato root rot nematode (*Ditylenchus destructor*) and the stubby root nematodes (*Trichodorus* and *Paratrichodorus* spp.) (Lima *et al.*, 2018). Not only do the nematodes significantly lower the yields, but they also distort the quality of potatoes leading to unmarketability (Mburu *et al.*, 2020). They cause physical and chemical changes to potato tubers thus reducing their shelf life, form lesions and malformations that render the produce unmarketable (Pinheiro *et al.*, 2015).

Despite the documented importance of potatoes to Kenya's food system, most pest management studies have focused on fungal and bacterial pathogens, with nematodes often overlooked. In particular, there remains limited field-based evidence on nematode suppression and yield recovery using sustainable or biodegradable delivery systems under Kenyan conditions.

2.1.2 Pea production in Kenya and disease constraints

Peas are the fourth most important legume crop produced worldwide after soybeans, peanuts and beans (FAOSTAT, 2024) (Fig. 2.2). Domesticated in 7000 BC, peas have since been grown for both human food and animal feed as they offer a good source of protein, starch, nutrients, vitamins, polyphenols and fibre (Wu *et al.*, 2018). Compared to other vegetables, they have high macronutrients (ascorbic acid, riboflavin and thiamine) and are rich in iron making them a nutritionally favourable legume crop (Martín-Cabrejas *et al.*, 2019). Peas are also suitable as forage, hay, silage and green manure as they have a high percentage of digestible nutrients (Infonet-Biovision, 2021). The use of peas as green manure leads to improve physical, chemical and biological soil properties and productivity (Chimouriya *et al.*, 2018). Peas are therefore considered an important crop as they contribute to food and nutrient security, poverty eradication and improvement of economy development.

Figure 2.2 Garden peas (shelled and unshelled in pods)
Source: Kumari and Deka (2021)

In 2009, the world production of peas was more than 10 million tonnes with Canada, China, USA and India as the major producers, in that order (Dahl *et al.*, 2012). By 2016, production had increased to 14.36 million tonnes according to FAOSTAT (2024). In Kenya alone, 82,000 metric tonnes were produced in 2016 from 20,416 ha grossing over KES 2,000,000,000 in revenue (KALRO, 2023). They are adapted to cool and moist climates (Wu *et al.*, 2018) and are mainly grown by smallholder farmers for domestic use and export. Temperatures ranging between 10 – 30ºC are recommended because above 30ºC, there will be poor pollination, early maturity and low yields (GoSA, 2021). Major pea growing areas include Kiambu, Nyandarua, Uasin Gishu, Laikipia, Nakuru, and Meru Counties (Royal Seedlings, 2024).

Despite having a potential of yielding up to 6 t/ha under optimum conditions, farmers in Kenya realize less than 4 t/ha due to a range of constraints (KALRO, 2023). These include limited knowledge of improved cultivars, poor soil fertility, poor crop management practices, lack of an organized marketing system, and pests and diseases. Major pests affecting peas in the field are pod borer *Etiella zinckenella* (*Maruca vitrata*), pea thrips (*Thrips tabaci, Frankliniella occidentalis*, *F. schultzeii* and *Ceratothripoides brunneus*), pea aphid (*Acrythosiphon pisum*), pea leaf miners (*Chrotomyia horticola*), Mexican bean beetle (*Epilachina varivestis*) and red spider mites (*Tetranychus* spp.) (Dhall, 2017). On the other hand, *Pseudomonas syringae* pv. *pisi*, Aphanomyces root rot, *Mycosphaerella pinodes*, fusarium wilt (*Fusarium oxysporum* f.sp. *pisi*), powdery mildew (*Erysiphe pisi*), and rust (*Uromyces viciae fabae*) are among the 32 important diseases (Coyne *et al.*, 2000; Prioul *et al.*, 2004; Dhall, 2017; Khulbe and Sharma, 2020). Soil-borne nematodes and weeds have recently been considered as a major cause of reduced yields of garden peas in Kenya (Infonet-Biovision, 2021).

The most important nematode pests of peas include *Meloidogyne* spp., *Pratylenchus* spp., *Heterodera* spp. (Riga *et al.* 2008). Overall, they cause wilting, yellowing, stunting, lesions and reduce the root's ability to fix nitrogen by distorting nodules (Inglis, 1998; Mellis, 2023). In as much as root exudates are known to cause hatching of some nematode eggs consequently leading to their multiplication, some studies have now shown the ability of pea root-cap exudates in inducing quiescence of plant parasitic nematodes (Hiltpold *et al.*, 2015; Jaffuel *et al.*, 2015).

Although nematode-induced yield losses in peas are recognized, studies quantifying their impact and exploring integrated nematode management in leguminous systems in Kenya are scarce. This limits understanding of how pea rotations or root exudates may influence nematode suppression.

2.2 Nematodes

Nematodes are worm-like microscopic organisms that are found in diverse environments including water, atmosphere and soil (Borgonie *et al.* 2011) (Figure 2.3). They are distributed worldwide (Agrios, 2005) where some have been found in caves (Du Preez *et al.*, 2015; 2017) while others are even surviving in space (Kaplan *et al.*, 2020). Majority of nematodes have specific requirements for soil and climate (Efthimiadou *et al.,* 2009; Yeates and Newton, 2009). Whereas most do best in well perforated sandy soils, a few favor clay soils such as the rice nematode (*Hirschimaniella* spp.) (Bridge *et al.*, 2005; Maung *et al.*, 2010).

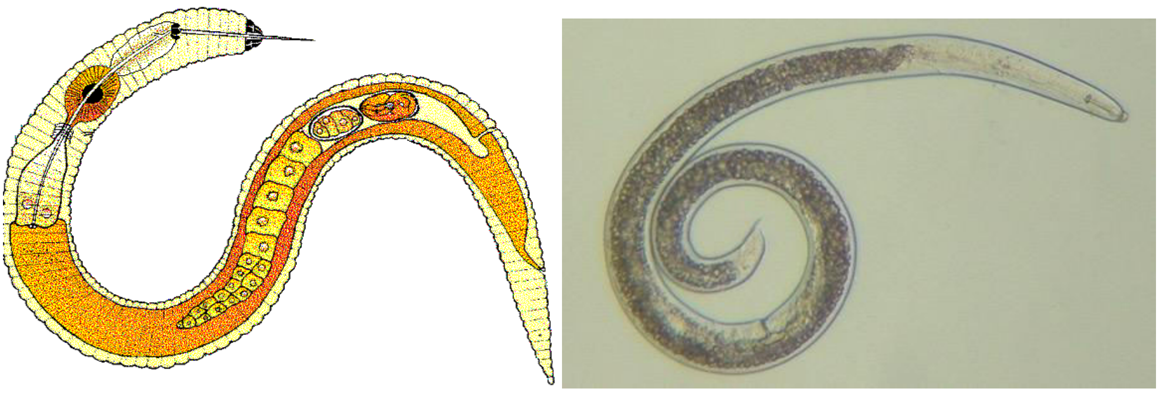

Figure 2.3 Typical worm-like nematode drawing (left), and as seen under microscope at x400 magnification (right)

Source: Coyne *et al*. (2018b)

Warmer regions appear to be a preference for more than half of the nematode species where their reproductive rates increase due to extended growing seasons (McSorley, 2011). The average life cycle of nematodes is 28 days under optimum conditions (Coyne *et al.*, 2014). Studies have shown that the life cycle can increase by up to fourteen days in colder regions (Carlsson *et al*., 2013). The most economically important nematodes are those found in the agroecosystems as they can have either positive or negative impact to soil health (Forghani and Hajihassani, 2020). Therefore, from a soil health perspective, nematodes are usually categorized as either plant parasitic nematodes (PPN) or free-living nematodes (FLN) (Topalović and Geisen, 2023).

2.2.1 Plant parasitic nematodes and their management

Most plant parasitic nematodes feed either inside or outside the host roots with the aid of a stylet which is a hollow mouth spear used in piercing and puncturing of plant cells (Atandi, 2018). They tend to cause the greatest damage when they attack seedling roots immediately after germination (Sikder and Vestergard, 2020). Their feeding oftentimes decreases the host's ability to absorb water and nutrients thereby resulting in stunted growth or plant death in severe cases (Coyne *et al.*, 2014). Endoparasitic nematodes which feed within the plant tissues appear to cause more damage than their ectoparasitic counterparts as reported by Perrine-Walker (2019) who suggested that a single endoparasite could kill a plant whereas hundreds of ectoparasites may feed on a plant without reducing productivity. Examples of endoparasitic nematodes includes sedentary nematodes such as root knot nematodes (*Meloidogyne* spp.), cyst nematodes (*Heterodera* spp. and *Globodera* spp.) (Fig. 2.4) and migratory nematodes such as the root-lesion nematodes (*Pratylenchus* spp.) and burrowing nematodes (*Radopholus* spp.) (Greco and Inserra *et al.*, 2024). Ectoparasitic nematodes normally feed externally through the plant cell walls and they include the spiral nematodes (*Helicotylenchus* spp., *Hoplolaimus* spp. and *Rotylenchus* spp.), ring nematodes (*Criconema* spp. and *Criconomella* spp.) among others.

Figure 2.4 Potato cyst nematodes on plant roots and under a microscope

Source: Adapted from Eurofins Agro (2024)

Through their feeding and damage caused by migration, PPN cause injuries to plants thereby facilitating entry and infestation by other soil-borne pathogens like bacteria and fungi (Kantor *et al.*, 2024) (Fig. 2.5). Moreover, some PPN have been shown to form disease complexes with the secondary pathogens which could result in total crop failure (Lopez-Nicora *et al.*, 2025). Examples include root knot nematode-bacterial wilt complex (RKNBW), potato cyst nematode-bacterial wilt complex (PCNBW) on solanaceous and Fusarium wilt-lesion nematode complex (Ravichandra, 2013; Khan and Sharma, 2020; Parrado and Quintanilla, 2024). A few PPN are known to transmit important plant viruses with the aid of a specialized stylet (onchiostylet or odontostylet). *Xiphinema* spp. and *Longidorus* spp. are known to transmit nepoviruses such as tomato leaf spot virus and raspberry ringspot virus, respectively whereas *Trichodorus* spp. and *Paratrichodorus* spp. transmit tobraviruses such as tobacco rattle virus and corky ringspot of potato (Spraing), respectively (Decraemer *et al.*, 2024).

Figure 2.5 A comparison between healthy (left) and nematode-damaged (right) potatoes

Despite causing an estimated worldwide loss of US $ 157 million in crop production (Abad *et al.*, 2008), plant parasitic nematodes were often overlooked in the past as important pests owing to their microscopic nature and lack of capacity (Cortada-Gonzales *et al.*, 2020). Furthermore, the symptoms they cause in the field are similar or identical to those caused by other biotic or abiotic factors e.g., wilting, stunting, patchy growth (Coyne *et al.*, 2018b). For example, potatoes that have been infected by *Globodera* spp. (potato cyst nematode) show yellowing symptoms which strongly resembles water or nutrient deficiency (Lima *et al.*, 2018). Knowledge of their damaging effects has become increasingly available especially in developed countries; however, this is not the case in sub-Saharan Africa (Cortada-Gonzales *et al.*, 2020).

2.2.1.1 Chemical management of nematodes

Chemical control of nematodes dates back to the 1940s and relies on the use of pesticides with nematocidal properties (Allen and Raski, 1950). Before 1940, Carbon disulphide was commercialized as a nematicide against *Heterodera* spp. and *Meloidogyne* spp. (Bessey, 1911) and Chloropicrin was considered an effective nematicidal chemical in the soil with limited data available (Ul Haq *et al.*, 2020). Presently, nematicides continue to be an important management strategy whether they are used alone or as part of an integrated pest and disease management program (Faske and Hurd, 2015). Over the decades, numerous studies have shown the correlation between nematicide application and increase in crop yields while citing plant parasitic nematodes as a high-risk pest group (Quénéhervé *et al.*, 1991; Duncan, 2009; Perry and Moens, 2013; Oka, 2020).

The nematicides, however diverse they can be in their chemical and biological activities, are generally categorized into two groups: fumigants and non-fumigants (Liu *et al.*, 2022). Chemically, they can be categorized as halogenated aliphatic hydrocarbons, methyl isothiocyanate liberators, organophosphates and organocarbamates (Ntalli and Caboni, 2017). Fumigant nematicides are highly effective in the reduction of nematode populations but they are too costly and require specialized application skills (Oka, 2020). Examples of fumigants include Chloropicrin, Methyl bromide, 1,3 Dichloropropene and Ethylene dibromide. On the other hand, non-fumigant nematicides which are more common than fumigants, are far less expensive and are more systemic in nature. The group is made up of organophosphates (e.g., fensulfothion, fenamiphos, ethoprophos, terbufos, cadusafos, fosthiazate, and imicyafos) and organocarbamates (aldicarb, fenamiphos, carbofuran and oxamyl) (Watson and Desaeger, 2019; Oka, 2020). They were formulated as granules or liquids to ease application methods and lower the quantity of applications (Yun, 2012; Abd-Alla *et al*., 2013). Unfortunately, their high toxicity to mammals and lack of specificity in their toxicity are a major drawback that has led to constant criticism (Gupta *et al.*, 2011; Jones *et al.*, 2017).

The overall use of chemical pesticides in management of pests and diseases has often been discouraged due to high toxicity (Poveda *et al.*, 2020), high cost (Talavera-Rubia *et al*., 2022), resistance development (Chen *et al*., 2020), mammalian toxicity (Tiwari, 2024) and environmental pollution (Wang *et al*., 2024). This has consequently led to banning or withdrawal of various formulations of nematicides across different countries such as methyl bromide and 1, 2 D, and the restricted use to liquid formulations (Abd-Elgawad and Askary, 2018; Oka 2020). Currently, there is no systemic nematicide that has been developed that can be applied safely to plants killing endoparasitic nematodes (Chen *et al.*, 2020). In spite of the limitations, it is said that the judicious use of nematicides may be warranted and even economical on selected high value crops (e.g., grapes, potatoes, vegetables and cotton) in heavily infested soils. Moreover, a number of studies have shown that using low-toxic nematicides at acceptable doses is still effective in reducing the population densities of plant parasitic nematodes on numerous crops (Ochola *et al.*, 2020; Pirzada *et al.*, 2020). Current nematode management strategies remain heavily reliant on synthetic nematicides despite growing restrictions and environmental concerns. There is a lack of research comparing chemical and biological agents under smallholder field conditions.

2.2.1.2 Biological management of nematodes

Biological control agents have been widely utilized in the management of plant pests and diseases but less on plant parasitic nematodes (Abd-Elgawad and Askary, 2018; Pires *et al.*, 2022). Their importance as bio-nematicides is growing rapidly as reflected by the considerable capital invested in their research (Wilson and Jackson, 2013). Microorganisms found to be involved in nematode suppression are fungi (predatory fungus, wireworm parasitic fungus, egg parasitic fungus, poisonous fungus and mycorrhizal fungus) (Zhang *et al.*, 2017a; 2020), bacteria (Gamalero and Glick 2020; Migunova and Sasanelli, 2021), and protozoa (Rønn *et al.* 2012). The general mode of action usually involves inducing resistance or direct parasitism or antibiosis (Poveda *et al.*, 2020).

Fungal microorganisms like *Trichoderma* spp., *Purpureocillium* spp., *Paecilomyces* spp., *Verticillium chlamydosporium*, *Hirsutella rhossiliensis*, and bacteria like *Pausteria penetrans* and *Bacillus* spp. (Schouteden *et al.*, 2015) have been extensively explored. Endophytic fungi are known to colonize plant roots whereby they offer benefits such as enhanced protection against biotic and abiotic stresses (Van Dessel *et al.*, 2011). *Paecilomyces lilacinus* and *Pochonia chlamydosporia* have even been tested in the field for the control of nematodes (Moreno-Gavíra *et al*., 2020). However, *P. lilacinus* has limited effectiveness as a microbial agent because of the correlation between rate of infection and duration of injection (Kergunteuil *et al.*, 2016; Ansari *et al.*, 2020). On the other hand, *P. chlamydosporia* which usually infects eggs and females and has the ability to colonize the plant rhizosphere (Zhang *et al.*, 2017b) but varies with plant species and is ineffective in infecting the eggs of root nematodes.

Although biological control agents such as *Trichoderma* spp. have shown promising potential for nematode suppression, most existing studies are limited to greenhouse conditions or short-term pot experiments. There is still insufficient field-based validation of their consistency, persistence, and interactions with native soil biota under Kenyan environments. Furthermore, little is known about how biological agents perform when delivered through biodegradable carriers or integrated with resistant cultivars. This gap highlights the need for long-term, systems-level research to evaluate biological control within holistic nematode management frameworks that balance efficacy, cost, and ecological sustainability.

2.2.1.3 Integrated nematode management ("Banana fibre paper technology")

Banana fibre paper technology is an innovative climate-smart technique that involves the use of treated banana paper on planting materials (seeds/tubers/suckers/vines) for management of PPN (NCSU, 2015). The technology involves treating banana fibre paper with micro-dosages of pesticides (as low as a thousandth of the recommended rate). This technique was considered as a method of controlled release of pesticides since approximately 10% of pesticides are available to plants while the rest gets lost in soil, water or air consequently leading to environmental pollution (Baweja *et al.*, 2020; Bondareva and Fedorova, 2021).

The aim was to present a cost-effective yet sustainable approach for crop protection through the use of biodegradable seed wraps produced from banana waste (Pirzada *et al.*, 2020). A number of plants have been analyzed for their sorption and release properties including rice husks, sawdust, jute, *Moringa oleifera* and banana fibre (Bello *et al.*, 20120). Cao *et al.* (2016) tested abaca, softwood, hardwood and banana fibre for this technology. Banana fibre paper was confirmed to be the most effective matrix as it contained high levels of cellulose content (44-54%) and low amount of lignin (6-13%) (Pirzada *et al.*, 2023). Moreover, the high availability of bananas worldwide generates abundant waste that can be effectively used for paper production, making bananas the most suitable for mass production.

Figure 2.6 From top left to bottom right: Banana fibre paper, mass treatment of paper with chemical nematicide, and potato tuber wrapped with banana paper before planting
Source: Cortada (2022)

The mode of action encompasses: 1) physical control where the banana fibre paper acts as a physical barrier to prevent nematodes from reaching and attacking the host roots; 2) adsorption whereby the paper adsorbs the hatching factors (root exudates) released by the host and therefore prevents cyst nematodes from hatching (Ochola *et al.*, 2022) chemical control in which the nematicides present in the paper, abamectin or fluopyram, act by causing nematode paralysis and blocking nematode cellular respiration thus affecting motility, respectively and;

4) biological control where the *Trichoderma asperellum* in the paper directly parasitizes eggs and juveniles of nematodes while indirectly boosting vegetative growth through root and soil colonization.

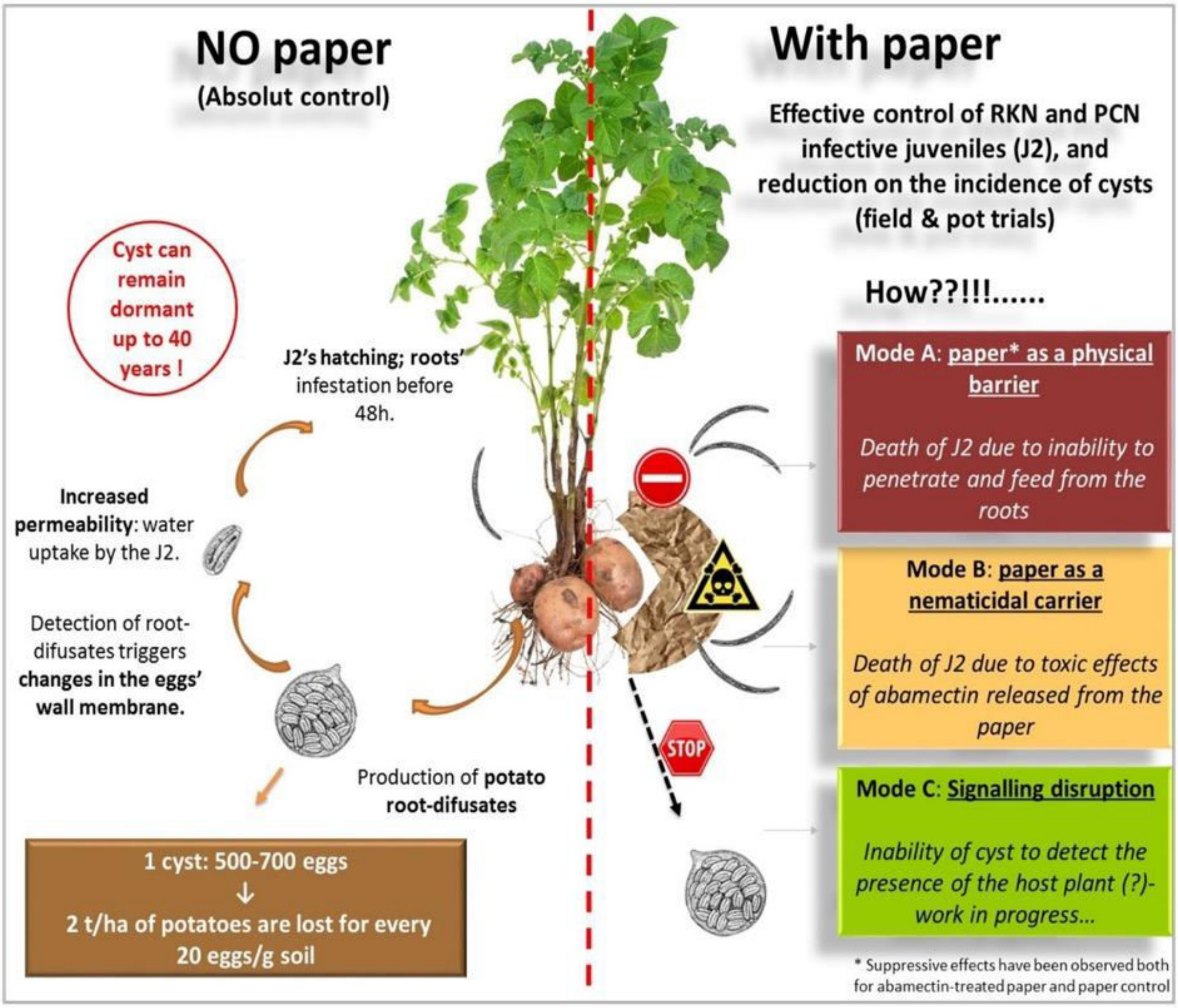

Figure 2.7 Pictorial demonstration of mechanisms behind banana fibre paper

Source: Cortada (2022)

So far, the technology has been successfully used in improving abamectin efficacy for root-knot nematode control in tomatoes (*Solanum lycopersicum*) (Cao *et al.*, 2016). Affokpon *et al.* (2018) used abamectin-treated banana paper to reduce densities of *Scutellonema braidys* (yam nematode) and other plant parasitic nematodes by up to 85% in seed yams (*Discorea alata*) under field conditions in West Africa. In East Africa, research on the use of abamectin-treated banana fibre paper on several crops including potatoes (*Solanum tuberosum*) and sweet potatoes (*Ipomoea batatas*) has shown promising results in regards to reduced nematode reproduction and increased yields (Cortada *et al.*, 2018; Ochola *et al.*, 2018). In potatoes, the technology entails selection and wrapping of average-sized sprouting seed tubers with banana fibre paper during planting. Following outcomes from extensive research on various crops, treated banana fibre paper has shown potential against PPN and in favor of improved crop yields (Kamau *et al*., 2024).

Despite encouraging proof-of-concept trials demonstrating that banana-fibre seed wraps can reduce nematode reproduction and improve yields (Ochola *et al*., 2022; Kamau *et al*., 2024), several important limitations remain in the literature. Most published studies have been short-term, single-site experiments that fail to capture inter-seasonal and agroecological variability, limiting their applicability across Kenya's diverse production zones. Reported outcomes are often restricted to plant parasitic nematode counts and yield, with limited consideration of non-target soil organisms such as free-living nematodes and microbial communities that are critical for soil health and ecosystem resilience. Comparative assessments between direct nematicide applications and matrix-incorporated delivery systems are also scarce, particularly under field conditions. Moreover, the performance of these biodegradable carriers in legume crops such as peas remains under-researched, despite their potential role in rotational nematode management. These gaps underscore the need for integrated, multi-season field evaluations that link nematode suppression, soil ecological responses, and agronomic outcomes across contrasting environments (Sasanelli *et al*., 2021).

2.2.2 Beneficial free-living nematodes

While plant parasitic nematodes (PPN) are widely recognized for their detrimental impact on crop productivity and soil health, the majority of nematodes in agricultural soils are actually free-living nematodes (FLN), comprising over 70% of the total nematode community (Andrássy, 2009). These non-parasitic nematodes play essential roles in maintaining soil ecosystem functions and are increasingly valued as indicators of soil health and ecological balance. Since their initial classification by Yeates *et al*. (1993), FLN have been categorized based on feeding behaviour into four principal trophic groups: bacterivores (which feed on soil bacteria), fungivores (which feed on saprophytic fungi), omnivores, and predators. Bacterivores and fungivores tend to be the most abundant in cultivated soils, whereas omnivores and predators are less commonly encountered but serve critical ecological functions (Kudrin *et al*., 2015; Kane *et al*., 2023; Zhang *et al*., 2024). Among the commonly reported bacterivores are members of the Cephalobidae (e.g., *Cephalobus*, *Eucephalobus*, *Cervidellus*) and Rhabditidae (e.g., *Rhabditis*, *Acrobeles*).

Beyond trophic classification, FLN are also functionally distinguished through life-history traits using the colonizer–persister (cp) scale developed by Bongers (1990). This classification reflects the ecological strategies of nematode taxa and their response to environmental stability or disturbance. At one end, cp 1 groups consist of r-strategists which are fast-reproducing, highly tolerant nematodes that dominate in disturbed soils. At the other end, cp 5 groups comprise K-strategists which are long-lived, stress-sensitive taxa associated with mature, undisturbed ecosystems. This ecological scaling underpins the Maturity Index (MI), an index widely used in nematology and soil ecology to infer successional dynamics and soil health (Ferris *et al*., 2001; Neher, 2010). Integrating trophic and life-history traits into a "functional guild" matrix has allowed for nuanced interpretation of nematode community responses to land management and environmental pressure (Ferris *et al*., 2001; van den Hoogen *et al*., 2020).

The ecological significance of FLN extends well beyond mere presence. These organisms actively mediate nutrient cycling, especially nitrogen mineralization, by stimulating microbial turnover through grazing (Neher *et al*., 2014). The abundance and diversity of bacterivores and fungivores have been linked to enhanced microbial activity, nitrogen cycling, and soil productivity (Sánchez-Moreno *et al*., 2010; Sieriebriennikov *et al*., 2014). Their contributions to soil fertility make them indispensable to sustainable agroecosystems. Furthermore, some groups of FLN are directly beneficial in pest management. Entomopathogenic nematodes such as *Heterorhabditis* spp. and *Steinernema* spp., which engage in mutualistic associations with insect-pathogenic bacteria, have been commercialized as biocontrol agents against insect pests (Adams *et al*., 2006; Denno *et al*., 2008; Saeedizadeh and Niasti, 2020). Similarly, FLN predators such as *Diplogaster* spp. and other mononchs have shown promise in suppressing populations of PPN (Bilgrami, 2008; Khan and Kim, 2007; Abd-Elgawad and Askary, 2018).

Another critical dimension of free-living nematodes (FLN) lies in their value as bioindicators of soil quality and ecosystem disturbance (Neher and Darby, 2009). Their broad trophic diversity, cosmopolitan distribution, and rapid response to management and environmental changes make them sensitive and integrative indicators of soil ecological condition (Du Preez *et al*., 2018; Lazarova *et al*., 2021; Zhao *et al*., 2024). Unlike chemical soil tests that provide static snapshots, nematode community structures reflect both current and cumulative effects of agricultural practices, offering insight into nutrient cycling, food-web dynamics, and soil resilience. Their responsiveness to tillage, fertilization, and organic amendments has positioned them as a robust diagnostic tool in soil health frameworks across both temperate and tropical systems (Neher, 2010; van den Hoogen *et al*., 2020).

The conceptual foundation for nematode-based soil assessment lies in faunal analysis frameworks such as that developed by Ferris *et al*. (2001), which use ecological indices to interpret nematode assemblages in functional terms. Key among these are the Maturity Index (MI), Enrichment Index (EI), Structure Index (SI), Channel Index (CI), and Basal Index (BI).

These indices integrate the colonizer–persister (c-p) scale, representing life-history strategy and ecological stability, with feeding guild composition to infer soil conditions. The Maturity Index, for instance, provides an indication of successional stage: low MI values typically occur in disturbed or nutrient-enriched soils dominated by r-strategists (bacterivores and fungivores), while high MI values are characteristic of mature, undisturbed systems with complex trophic networks (Bongers, 1990; Ferris *et al*., 2001).

Similarly, the Enrichment Index (EI) quantifies the system's response to resource availability, often increasing rapidly following organic matter inputs that stimulate bacterial activity. A high EI therefore signifies active nutrient turnover and microbial enrichment, while a low EI points to nutrient exhaustion or depletion. The Structure Index (SI), in contrast, represents the degree of food-web maturity by emphasizing higher trophic levels such as omnivores and predators; low SI values signal food-web degradation or stress, whereas high SI values indicate functional redundancy and ecological resilience. The Channel Index (CI) distinguishes decomposition pathways, differentiating between bacterial- and fungal-dominated systems: low CI values reflect bacterial mineralization typically associated with fresh organic inputs, while high CI values suggest fungal-driven decomposition of more recalcitrant organic matter. The Basal Index (BI) captures stress-tolerant nematode guilds that dominate under conditions of chronic disturbance, toxicity, or low resource quality, hence serving as an indicator of persistent environmental stress (Neher, 2010; Du Preez *et al*., 2018).

Recent methodological advances have expanded this framework to include nematode metabolic footprints, which quantify the biomass and functional contributions of different trophic groups to ecosystem processes such as carbon flow and nutrient mineralization (Ferris, 2010; Sieriebriennikov *et al*., 2014; van den Hoogen *et al*., 2020). These approaches, implemented through computational tools such as NINJA, allow for simultaneous calculation of composite indices and footprints, providing a multidimensional assessment of soil function without requiring exhaustive taxonomic identification. The resulting data offer complementary insights: the EI reflects short-term nutrient responsiveness, the MI and SI indicate long-term structural stability, while metabolic footprints reveal energy flux and functional capacity within the soil food web (Lazarova *et al*., 2021).

Integrating these indices provides a powerful means to evaluate the ecological sustainability of nematode management strategies. By analyzing shifts in free-living nematode communities, researchers can assess whether interventions, such as chemical nematicides, biological inoculants, or biodegradable paper matrices, achieve pest suppression without compromising soil ecological integrity. This functional perspective is particularly valuable in systems where nematicide use risks disrupting beneficial soil biota. Studies combining PPN suppression data with nematode faunal analyses have demonstrated that sustainable management practices maintain high EI and moderate-to-high SI values, reflecting both nutrient enrichment and preserved trophic complexity (Du Preez *et al*., 2018; Zhao *et al*., 2024). Thus, nematode-based indices bridge the gap between pest control and soil ecosystem health, supporting the development of integrated, ecologically balanced nematode management frameworks.

In this study, FLN were analysed both as non-target taxa affected by nematicidal interventions and as functional indicators of soil health. By evaluating shifts in trophic composition, enrichment and structural indices, and colonizer–persister profiles, the ecological consequences of banana paper treatments could be assessed holistically. This approach reflects a growing emphasis on biologically integrated pest and soil health management, where FLN serve not only as passive indicators but as active agents of agroecosystem sustainability.

CHAPTER THREE: GENERAL METHODOLOGY

3.1 Field sites

Several field trials were conducted in Nyandarua county (0.1804° S, 36.5230° E) (Appendix iv) owing to its high potato production and the prevalence of key nematode pests. An additional trial location was set up in Nyamira county (0.6483° S, 34.9948° E) (Appendix v) to evaluate the effect of banana paper on nematode occurrence and distribution in the western highlands of Kenya. According to FAO-UNESCO (2003), the soils in Nyandarua have been described as clay-loam volcanic with moderate to high soil fertility while Nyamira has red volcanic nitosols which are excellent for farming. Table 3.1 highlights the climatic characteristics of the study areas while Appendix vi shows the rainfall and temperature during the study period.

Table 3.1 General climatic and geographical characteristics of the study locations

	Nyandarua	Nyamira
AEZ	Upper Highland 1 (UH1)	Lower Midland 2 (LM2)
Rainfall	700-1600 mm	1200-2100 mm
Temperature	12-25^{o}C	10.1 – 28.7^{o}C
Altitude	2400-3999 m	1877 – 2191 m
Longitude	35° 13‘E	35° 0′ E
Latitude	0°50‘S	0° 46′ S
Long rains	Feb – July	Feb – July
Short rains	Aug – Dec	Aug – Dec

Source: Council of Governors (2013) and County Government of Nyamira (2018)

3.2 Banana fibre paper treatments

During the study, three treated banana fibre papers were used: abamectin-treated paper (chemical based), fluopyram-treated paper (chemical based) and *Trichoderma*-treated paper (biological based). The rates of application of the nematicides applied are shown in Table 3.2 below which utilized a micro-dosage, less than one ten-thousandth ($<1\times10^{-4}$) of the recommended chemical nematicide rate, compared to the standard application rates of 160 g ha^{-1} for abamectin and 250 g ha^{-1} for fluopyram. These rates were selected based on prior research and established effective microdosing standards: Abamectin rates followed those previously applied in field trials by Ochola *et al*. (2022), which demonstrated effective suppression of potato cyst nematodes at low doses when delivered through banana fibre paper. Fluopyram rates were adapted from guidelines developed by North Carolina State University (NCSU), where microdose applications of 0.03–0.05 g active ingredient per plant provided effective nematode suppression while minimizing environmental exposure (NCSU, 2018). By adopting these validated rates, the study ensured that chemical inputs were efficacious and sustainable, while allowing the banana fibre paper to function as a controlled, localized delivery system.

An untreated paper was included as a positive control, in which plain banana fibre paper was wrapped around the planting material prior to planting to account for any effects of the paper itself on nematode suppression or plant growth. In addition to the positive control, an absolute control was included, representing the typical farmer practice. In this treatment, planting material was left unwrapped, without any banana fibre paper or nematicide application. This would be referred to as control throughout the study.

Table 3.2 Rates of application of the banana paper treatments applied on potato and pea trials at Nyandarua and Nyamira counties, Kenya

Control agent	Treatment	Active ingredient	Concentration
Chemical	Abamectin-treated paper	Avermectin	100 ng/L*
Chemical	Fluopyram-treated paper	Fluopyram	10 ng/L*
Biological	*Trichoderma*-treated paper	*Trichoderma* asperellum	12 ml/L ha^{-1}
Physical	Untreated paper	Nil	Nil
None	Control (no paper)	Nil	Nil

The banana fibre paper, measuring 10 x 12.5 cm (Pirzada *et al.*, 2020), was supplied by North Carolina State University (NCSU), Raleigh, NC, USA. The abamectin- and fluopyram-treated papers were supplied pre-treated from NCSU.

3.3 Nematode assessment

Soil samples were collected from the experimental plots at both planting and harvesting stages using a cross-diagonal sampling pattern (Coyne *et al.*, 2018b). Topsoil was collected at a depth of 5-30 cm using a hand trowel. Multiple subsamples were taken from each plot, thoroughly mixed to form a composite sample of 2 kg and packaged in a ziplock bag. The composite samples were stored in cool boxes and transported to the *Nem*Africa laboratory at *icipe*, Kenya for further processing.

Nematode extraction for vermiform nematodes was conducted following the treated Baermann's technique from 100 ml of soil (Coyne *et al.*, 2014) within three days of sampling. After 48 hours, the nematode suspension was passed through a 25 µm sieve. Nematode density was then estimated by counting the total number of nematodes in each processed sample (Fig. 3.1). A known volume of the nematode suspension (2 ml) was taken using a pipette and placed on a De Grisse counting dish (Atandi, 2018). The sample was observed under a Leica MZ12 stereo dissecting microscope (Leica Microsystems, Wetzlar, Germany) at x20 magnification (Coyne *et al.*, 2014), and nematode counts were recorded using a hand-held tally counter.

Figure 3.1 Modified Baermann's extraction method (left) and nematode counting using a stereo microscope (right).

Nematodes were thereafter killed using the hot water bath method by rapid Seinhorst technique (Hooper, 1988). This involved placing vials which had nematode suspension into 80° C hot water for two minutes. The nematodes were then preserved by adding two-three drops of 4% formaldehyde (10% formalin) (Van Bezooijen, 2006). Nematodes were identified morphologically up to the genus level using a ZEISS Axiolab 5 compound microscope (Carl Zeiss NTS Ltd, Oberkochen, Germany) up to a magnification of ×400 (Coyne *et al.*, 2014) where the first 100 nematodes per sample were considered (Fig. 3.2). They were identified using morphological features as described by Siddiqi (2000), Luc *et al.* (2005) and the University of Nebraska Lincoln nematode identification website (KSU, 2023) (Appendix vii).

Figure 3.2 Nematode identification on a compound microscope

The primary features used to distinguish nematode genera included body size and shape, such as total length, width, and body tapering, which could be filiform, cylindrical, or clavate. Stylet type and size were also important, with nematodes possessing a stomatostylet, odontostylet, or onchiostylet, including measurements of length and basal knobs. Tail morphology was examined, noting whether it was round, filiform, conoid, or blunt, as well as the presence of hyaline regions. The position of the oesophagus–intestinal overlap, relative to the dorsal or ventral side, was considered for differentiating cyst and root-knot nematodes. Structures in the cephalic region, such as the presence or absence of probolae, lip region morphology, annulations, and labial disc, were used to further refine genus-level identification. Reproductive structures were examined in both sexes: in females, vulva position was expressed as a percentage of body length (10–30%, 40–60%, 70–90%), while in males, the presence or absence of a bursa and the shape of spicules were noted. Additional features, including the number of lateral field incisures, cuticle ornamentation, and the presence of phasmids, were also considered in distinguishing nematode genera.

For the extraction of the globe-shaped potato cyst nematodes (PCN), soil was first dried in the screenhouse for over 48 hours. Upon drying, 200 ml of soil was measured and passed through a Fenwick can to extract cysts (Coyne *et al.*, 2018b) as shown in (Fig. 3.3). The cysts were collected onto filter papers and allowed to dry for 24 hours. Under a Leica stereo microscope with above light, cyst nematodes were carefully picked using soft forceps, quantified with a tally counter then kept in 1.5 mm Eppendorf vials and properly labelled.

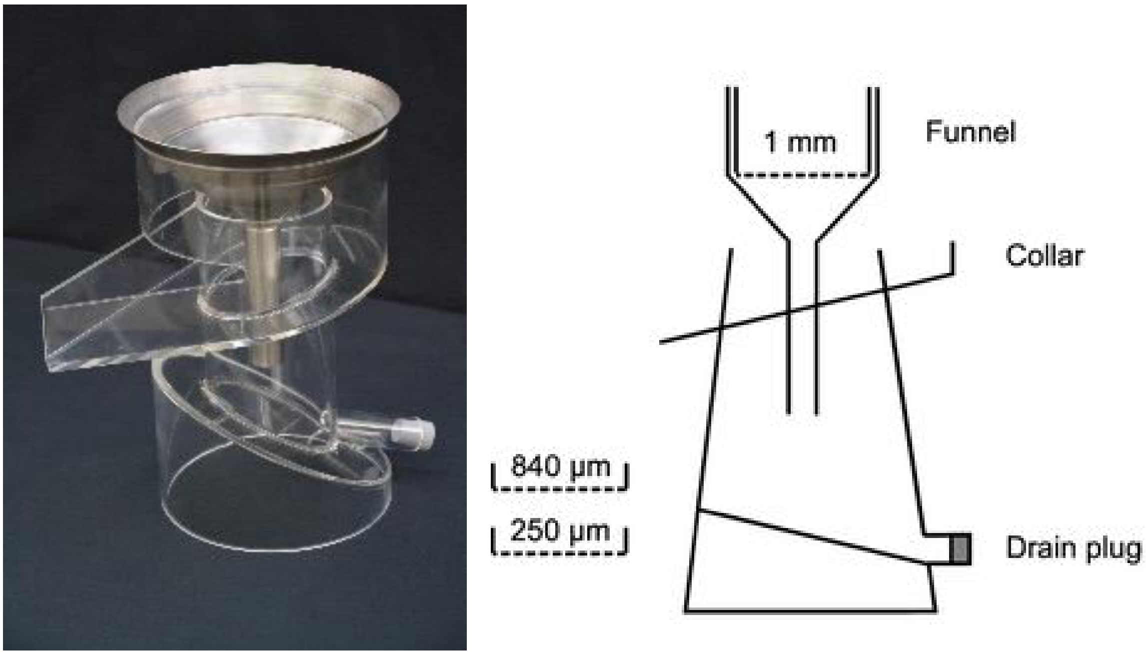

Figure 3.3 Standard Fenwick can for the extraction of cysts

3.4 Yield assessment

Data for potato yield was collected approximately 90 – 120 days post planting at crop maturity stage, depending on the potato cultivar. This involved counting the number of tubers and measuring their weight per plot (in kg). For quality assessment of, potato tubers were graded into two main categories: marketable and unmarketable yield. Marketable yield entailed healthy, undamaged tubers measuring at least 20 mm in diameter. Measuring was done using a string and a ruler. Unmarketable yield comprised small sized, damaged or rotten tubers (Fig. 3.4).

Figure 3.4 Comparison between marketable and unmarketable potato tubers

On the other hand, data for peas was collected at physiological maturity by manually plucking mature green pods from plants. In each plot, the total number of pods was measured using a digital weighing scale and standardized to t ha^{-1}. Visual grading was conducted immediately after harvest, with marketable pods defined as well-filled, fresh, and undamaged. Unmarketable pods were those with poor grain fill or physical damage from biotic or abiotic factors (Fig. 3.5).

Figure 3.5 Example of unmarketable pea pods

CHAPTER FOUR: NEMATODE CHARACTERIZATION UNDER CHEMICALLY TREATED BANANA FIBRE PAPER

4.1 Introduction

Abamectin and fluopyram are chemicals that were initially manufactured for the control of insects and fungal pathogens, respectively, but have now shown their nematicidal ability (Faske, 2009; Faske and Brown, 2019). Abamectin belongs to the group Avermectins (EFSA *et al*., 2017) that are macrocyclic lactones which have demonstrated high potential in the management of several insect pests, phytophagous mites and plant parasitic nematodes, specifically *Meloidogyne* spp. Currently, it is showing strong activity against a wide range of plant parasitic nematodes whereby it causes paralysis by binding to the nematode nerve and muscle cells (Liu *et al*., 2023; Massoud *et al*., 2023). Owing to its low toxicity to non-target soil organisms, abamectin has been accepted and incorporated in integrated pest and disease management programs (Khalil, 2013). Furthermore, it is readily degraded by soil microorganisms and does not persist in the soil or environment. However, it is unstable in light hence photodegrades rapidly and has low water solubility (Khalil *et al*., 2020; Cui *et al.*, 2022). With the introduction of nanotechnology, use of modern pesticide formulations like nanoparticles, nanosuspensions and nano-capsule pesticides against PPN may be the solution in application of abamectin (Sabry, 2019).

Fluopyram is of particular interest in cyst nematode management due to its systemic mobility, nematostatic action, and recent regulatory approval for use in vegetables. The mode of action involves the inhibition of succinate dehydrogenase (SDH) which is an enzyme found in prokaryotes that is responsible for metabolism and energy production (Oka and Saroya, 2019). Consequently, when organisms come into contact with fluopyram, they experience reduced motility hence are unable to move towards the food source and effectively die of starvation (Faske and Hurd, 2015). Root knot nematode eggs and juvenile densities were significantly reduced under fluopyram and ethoprophos nematicide application on lima beans (Jones *et al.*, 2017). However, Schleker *et al*. (2022) suggested that fluopyram exhibits efficacy differences among parasitic nematode species. The efficacy of fluopyram against *Globodera* spp. in open field conditions, particularly in sub-Saharan Africa, remains underexplored. Moreover, there is limited information on its effect when applied through alternative delivery platforms such as biodegradable paper matrices. This study therefore focused on evaluating abamectin- and fluopyram-treated paper as a novel control strategy against parasitic nematodes in potato production.

4.2 Materials and Methods

4.2.1 Study sites and design

Two separate field trials were conducted in Nyandarua county: Abamectin trials and Fluopyram trials to characterize PPN and assess the effect of chemically treated banana paper on potato yield. For abamectin trials, a multilocational research experiment was set up in the five subcounties of Nyandarua county. These locations were selected based on farmer groups within the subcounty and included Njabini (Kinangop), Geta (Kipipiri), Central (Ndaragwa), Charagita (Ol Joro Orok) and Rurii (Ol Kalou) (Table 4.1). A 2 × 2 factorial experiment was implemented within a split-plot design. Potato cultivars were assigned to the main plots, and banana fibre paper treatments to the subplots. Two potato cultivars, sourced from Jekam farm, Nyandarua, were tested: Shangi and Sherekea. The treatment factor involved comparing abamectin-treated paper to absolute control (farmer's practice).

Table 4.1 Coordinates of the field trial locations in Nyandarua county

Sub-county	Ward	Latitude	Longitude
Kinangop	Njabini	0.7516° S	36.6273° E
Kipipiri	Geta	0.4145° S	36.5986° E
Ndaragwa	Central	0.0581° S	36.5310° E
Ol-Joro Orok	Charagita	0.1603° S	36.2851° E
Ol Kalou	Kanjwiri	0.2261° S	36.3715° E

At each location, four plots measuring 5 x 5 m were established, where both potato cultivars were planted in two plots, with one plot receiving the abamectin-treated paper loosely wrapped around the potato seed tubers. Each plot fit 100 seed tubers spaced out at the standard recommended spacing of 30 cm between plants, 75 cm between rows, and a buffer zone of 0.75 m between plots. Each location (subcounty) served as a block. The trial was repeated over two cropping seasons during the long and short rain season with rainfall and temperatures as shown in Table 4.2 below.

Table 4.2 Weather details during the trial period

Season	Rain	Period	Rainfall (mm)	Mean temperature
Season 1	Long rains	Mar - May '21	578.67	17.23˚C
Season 2	Short rains	Nov '21 - Jan '22	357.23	17.10˚C

Seasonal rainfall collected during the study period
Temperature recorded daily and average between lowest and highest temperature

For fluopyram trials, a Type II experimental design (designed by researcher, managed by farmers) was conducted to assess the impact of fluopyram-treated banana paper on nematode abundance in Kinangop, Nyandarua county (-0.5589° S, 36.5752° E). Ten farmers were randomly selected, with each farmer considered as a replicate. To minimize variation in standard error, farmers were all within a 1 km radius. Each farmer managed two plots: one plot where fluopyram-treated paper was loosely wrapped around potato seed tubers and another untreated plot serving as the control. The popular Kenyan potato cultivar Shangi, sourced from Jekam Farm, Nyandarua, was planted at a rate of 100 tubers per plot with standard spacing resulting in a total plant population of 20,833 plants per hectare (Fig. 4.1).

Figure 4.1 Field trial set up showing a plot where potato tubers are wrapped in banana paper at planting.

A buffer zone of 1 m between treated and untreated plots was maintained. The experiment was carried out over three consecutive cropping seasons as depicted in Table 4.3 below to ensure consistency and reliability of the results.

Table 4.3 Weather details during the trial period

Season	Rain	Period	Rainfall (mm)	Mean temperature
Season 1	Long rains	Mar - May '21	578.67	17.23˚C
Season 2	Intermediate rains	Jul - Sep '21	169.85	16.11˚C
Season 3	Short rains	Nov '21 - Jan '22	357.23	17.1˚C

Seasonal rainfall collected during the study period
Temperature recorded daily and average between lowest and highest temperature

For both trials, routine agronomic practices were performed during each trial season. Di-ammonium phosphate (DAP) and calcium ammonium nitrate (CAN) were applied as planting and top-dress fertilizer, respectively, each at a rate of 50 kg/acre. Twigsgate (Glyphosate isopropyl ammonium 480 g/L) herbicide was applied twice each season at a rate of 2.5 L ha^{-1} to manage weeds. Ridomil gold (Metalaxyl-M 40 g/Kg + Mancozeb 640 g/Kg) at rate of 2.5 kg/ha was administered interchangeably with Infinito (Fluopicolide 62.5 g/L + Propamocarb hydrochloride 625 g/L) at a rate of 1.6 L/Ha as fungicides to manage fungal diseases which were prone during the rainy seasons. No irrigation was considered, and the trials remained strictly rainfed.

4.2.2 Nematode and yield evaluation

Nematodes were assessed as described is section 3.3 of Chapter three whereby composite soil samples were collected and processed immediately. Nematodes were extracted from 100 ml soil using the modified Baermann method (Coyne *et al*., 2018b), sieved through 25 µm, counted under a dissecting microscope and identified using morphological keys (KSU, 2023). Cyst nematodes were extracted from 200 ml soil using the Fenwick can.

Potato yield was collected at harvest time which is 90 days for cv. Shangi and 120 days for cv. Sherekea. Total yield was measured and quality assessed through marketable and unmarketable categories.

4.2.4 Data analyses

To determine the reproductive factor (RF) of nematodes, the RF was calculated using the equation below (Neher and Darby, 2009):

$$RF = \frac{Pf}{Pi}$$

where P_f = Final population; P_i = Initial population

For every season, the mean abundance of PPN was calculated from each treatment, cultivar and cropping season. All count data were presented as mean ± standard errors (Mean ± S.E.). Nematode data, cyst counts, and crop yield were tested for normality using Shapiro-Wilk test ($p > 0.05$) using GraphPad prism version 10.4.1 software (GraphPad Software Inc, San Diego, CA) (accessed 20 October 2023). Only data for PPN diversity was log transformed [Y= -1*log(Y)] to conform to assumptions of normality. Levene's test was additionally performed to test the homogeneity of variances.

Mixed model two-way ANOVA was performed to analyze the main and interaction effects of potato cultivar (resistant and susceptible) and treated banana fibre paper (abamectin-treated or fluopyram-treated) on PPN abundance and yield (marketable and unmarketable). Statistical significance was determined at the $p \leq 0.05$ level and when significant differences were observed, Tukey's honest significant difference (HSD) test was employed to separate means. All analyses were performed by R version 4.2.3 (R Core Team, 2023).

4.3 Results

4.3.1 Effect of abamectin-treated paper on plant parasitic nematodes abundance and diversity and yield

In total, thirteen genera of PPN were identified across the study, with representation from eight nematode families. These included *Aphelenchoides* (Aphelenchoididae), *Aphelenchus* (Aphelenchidae), *Tylenchorhynchus* (Belonolaimidae), *Criconema* (Criconematidae), *Globodera* and *Meloidogyne* (Heteroderidae), *Helicotylenchus* and *Rotylenchus* (Hoplolaimidae), *Xiphinema* (Longidoridae), *Pratylenchus* (Pratylenchidae), *Trichodorus* (Trichodoridae), and *Filenchus* and *Tylenchus* (Tylenchidae). There were no significant variations between Shangi and Sherekea potato cultivars and therefore data were pooled together for cultivar (Appendix viii). Nematode genera varied significantly ($p < 0.001$) across the treatments in the second season. The composition of the nematode community was dominated by *Globodera* spp. which constituted 25.38% of the total population in the first season. *Aphelenchus* spp. and *Criconema* spp. were highly abundant during the second season (Table 4.4). Between abamectin-treated paper and control, no significant differences were observed on the abundance of plant parasitic nematode genera.

Table 4.4 Abundance of plant parasitic nematode genera in potatoes in Nyandarua county, Kenya

	Season 1		Season 2	
Genera	Control	Abamectin-paper	Control	Abamectin-paper
Aphelenchoides	0 ± 0 c A	0 ± 0 e A	6 ± 3 bc A	1 ± 1 c B
Aphelenchus	145 ± 76 a A	139 ± 41 ab A	34 ± 10 a A	23 ± 5 ab A
Criconema	0 ± 0 c A	0 ± 0 e A	35 ± 6 a A	41 ± 14 a A
Filenchus	55 ± 19 b A	76 ± 17 b A	39 ± 15 a A	22 ± 4 ab A
Globodera	122 ± 75 a A	251 ± 132 a A	0 ± 0 c A	0 ± 0 c A
Helicotylenchus	4 ± 4 c A	32 ± 14 c A	0 ± 0 c A	0 ± 0 c A
Longidorus	3 ± 3 c A	0 ± 0 e A	0 ± 0 c A	0 ± 0 c A
Meloidogyne	7 ± 7 c A	0 ± 0 e A	0 ± 0 c A	0 ± 0 c A
Pratylenchus	144 ± 117 a A	102 ± 80 ab A	0 ± 0 c A	0 ± 0 c A
Rotylenchus	0 ± 0 c A	0 ± 0 e A	4 ± 3 bc A	1 ± 1 c A
Trichodorus	4 ± 3 c B	50 ± 31 bc A	27 ± 8 ab A	17 ± 4 b A
Tylenchorhynchus	3 ± 2 c A	6 ± 5 d A	0 ± 0 c A	1 ± 1 c A
Xiphinema	5 ± 5 c A	18 ± 16 cd A	0 ± 0 c A	1 ± 1 c A

Data represents pooled average of two potato cultivars presented per 100 ml soil.
Season 1 is long rains from March to May 2021 and season 2 is short rains from Nov 2021-Jan 2022.
Means followed by the same lowercase letters in each column grouping are not significant at $p < 0.05$
Means followed by the same uppercase letters across rows in each season grouping are not significant at $p < 0.05$

For mean nematode abundance, across both cropping seasons, there were no statistically significant differences between Shangi and Sherekea cultivars ($\chi^2 = 0.04$, $p = 0.83$) (Fig. 4.2). Likewise, nematode population densities did not differ significantly between abamectin-treated paper and control ($\chi^2 = 0.43$, $p = 0.51$), and no interaction effects between cultivar and treatment were detected ($\chi^2 = 0.03$, $p = 0.86$).

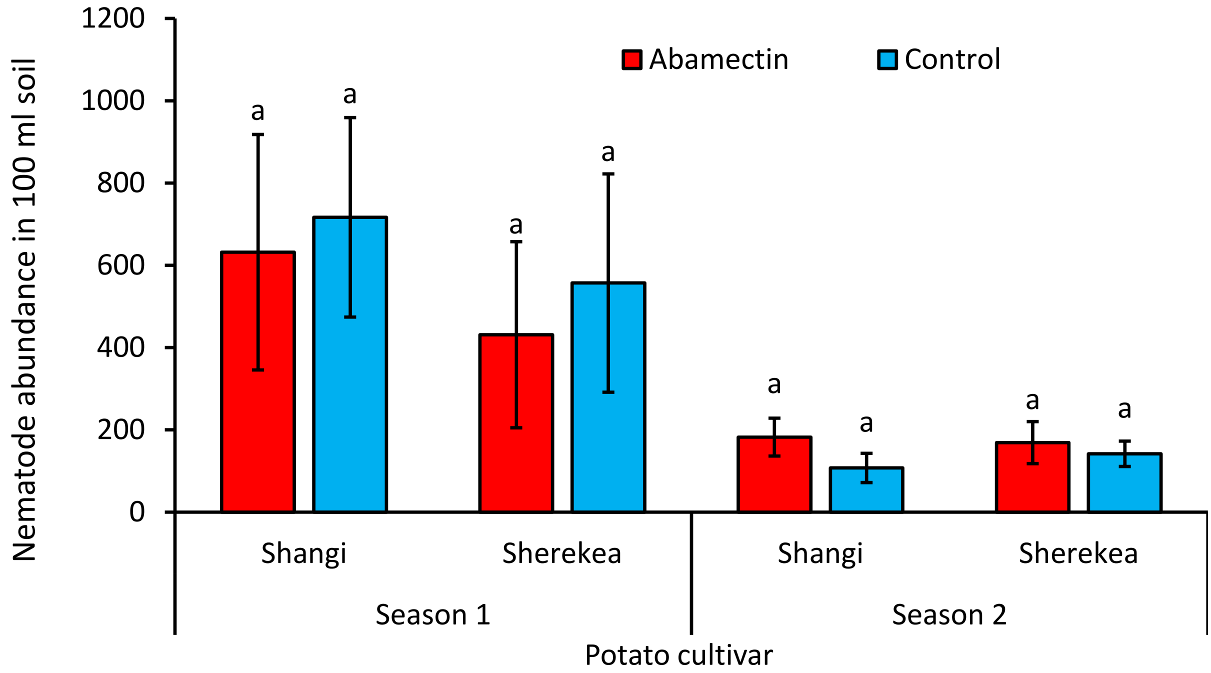

Figure 4.2. Effect of abamectin-treated paper on population densities of nematodes under potatoes Nyandarua county, Kenya.

Season 1 = long rains (Mar-May 2021); Season 2 = short rains (Nov '21-Jan '22). Within each potato cultivar, bars followed by the same letter are not significantly different at p < 0.05.

The reproductive factor (RF) of cyst nematodes was significantly influenced by season, potato cultivar, and treatment, with strong evidence of two- and three-way interaction effects. There were significant main effects of season ($\chi^2 = 207.78$, $p < 0.001$), cultivar ($\chi^2 = 63.86$, $p < 0.001$), and treatment ($\chi^2 = 106.65$, $p < 0.001$). Additionally, all interactions, season × cultivar ($\chi^2 = 47.06$, $p < 0.001$), season × treatment ($\chi^2 = 97.78$, $p < 0.001$), cultivar × treatment ($\chi^2 = 69.99$, $p < 0.001$), and the three-way interaction ($\chi^2 = 70.35$, $p < 0.001$), were statistically significant, indicating that treatment effects varied across seasons and potato cultivars.

In the first season, RF in Shangi were not significantly different between treatments (Control = 10.87; Abamectin = 7.32; $p > 0.05$) (Fig. 4.3). In contrast, Sherekea exhibited a significant ($p < 0.05$) reduction in RF under abamectin-treated plots (7.58) compared to control plots (38.96). In the second season, both cultivars exhibited reduced RF under abamectin-treated paper relative to the control, but these differences were statistically similar.

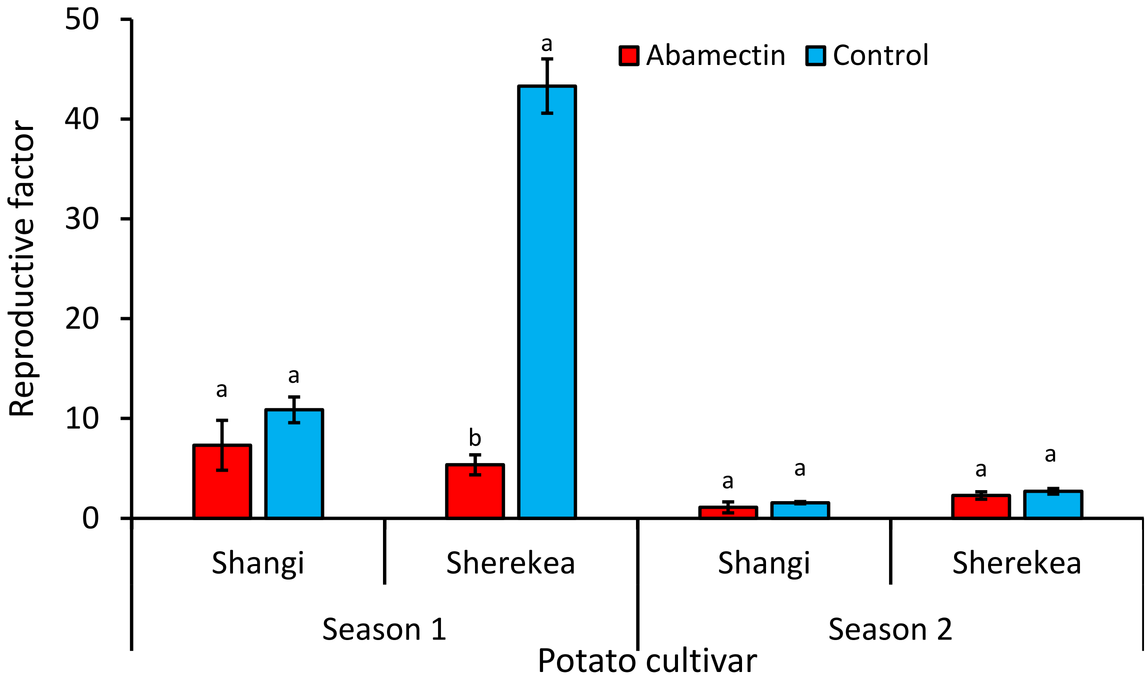

Figure 4.3. Effect of abamectin-treated paper on reproductive factor of nematodes under potatoes (cvs. Shangi and Sherekea) in Nyandarua county, Kenya.

Season 1 = long rains (Mar-May 2021); Season 2 = short rains (Nov '21-Jan '22). Within each potato cultivar, bars followed by the same letter are not significantly different at p < 0.05.

Application of abamectin-treated paper significantly ($\chi^2 = 24.74$, $p < 0.001$) increased total yield of potatoes for both Shangi and Sherekea cultivars compared to the control. Yield data, however, did not vary significantly between seasons ($\chi^2 = 3.0$, $p < 0.08$) and was therefore pooled for the two cropping seasons (Appendix viii). In Shangi, the total yield under abamectin-treated paper averaged at 13 t/ha, significantly higher than the control yield of 9 t/ha. Similarly, Sherekea recorded the highest total yield under abamectin-treated paper (16.5 t/ha) compared to the control (12.5 t/ha), with statistically significant differences as depicted in Fig. 4.4 below. Between the cultivars, Sherekea recorded significantly higher (χ^2 = 14.28, p < 0.001) yields than Shangi for both treatments. In contrast, no significant differences were observed for yield quality as the marketable yield between abamectin-treated paper and control remained similar for either cultivar.

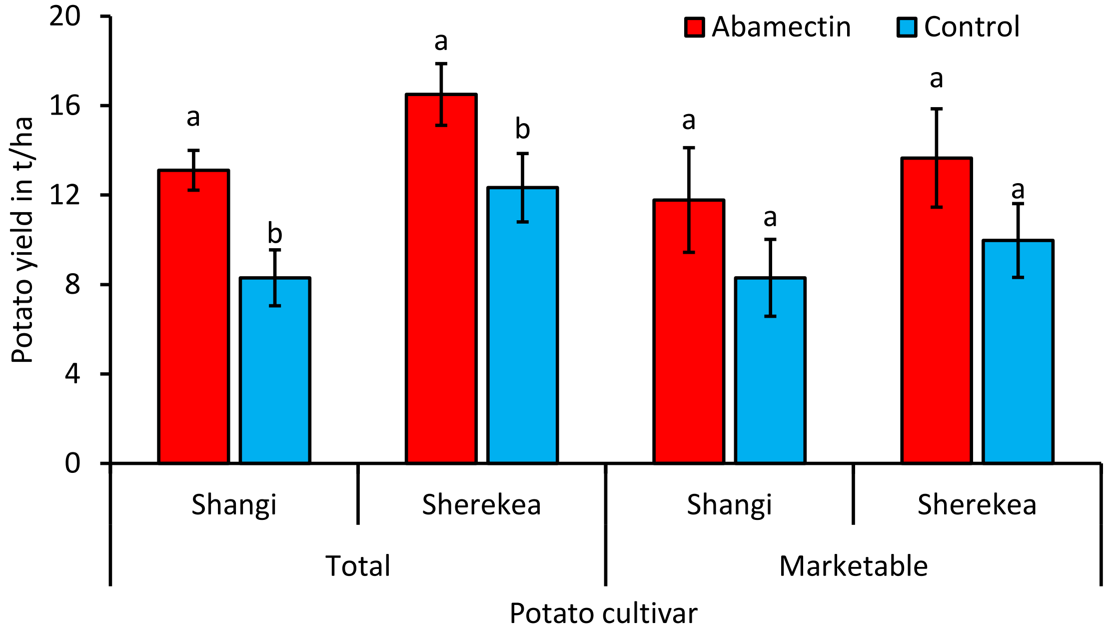

Figure 4.4. Effect of abamectin-treated paper on yield of potatoes (cvs. Shangi and Sherekea cultivars) in Nyandarua county, Kenya.

*Season 1 = long rains (Mar-May 2021); Season 2 = short rains (*Nov '21-Jan '22*). Within each potato cultivar, bars followed by the same letter are not significantly different at p < 0.05.*

4.3.2 Effect of fluopyram-treated paper on plant parasitic nematodes abundance and diversity and yield

During the initial planting stage (Pi) of each cropping season, the population densities of plant parasitic nematodes in fluopyram-treated plots and control plots were statistically similar ($\chi^2 \geq 0.01$, $p = 0.94$), indicating no initial differences in nematode densities across these treatments. However, when comparing across seasons, marked differences in nematode densities were observed. Notably, season 1 exhibited significantly higher nematode populations (>595 nematodes per 100 ml of soil), whereas season 3 recorded generally lower densities (<372 nematodes per 100 ml of soil) ($\chi^2 = 7.63$, $p = 0.02$).

Three months later, at the time of harvest (Pf), a significant treatment effect on nematode densities was observed ($\chi^2 = 59.31$, $p < 0.001$). Across all seasons, fluopyram-treated plots consistently recorded lower nematode densities (616, 281, and 567 nematodes per 100 ml of soil for seasons 1, 2, and 3, respectively) compared to the control plots, which exhibited densities of 1301, 649, and 567 nematodes per 100 ml of soil in the corresponding seasons. Furthermore, the nematode reproductive factor (RF) varied significantly between treatments within each season ($\chi^2 \geq 13.2$, $p < 0.01$), with fluopyram-treated plots showing approximately half the RF values of the control plot (Fig. 4.5).

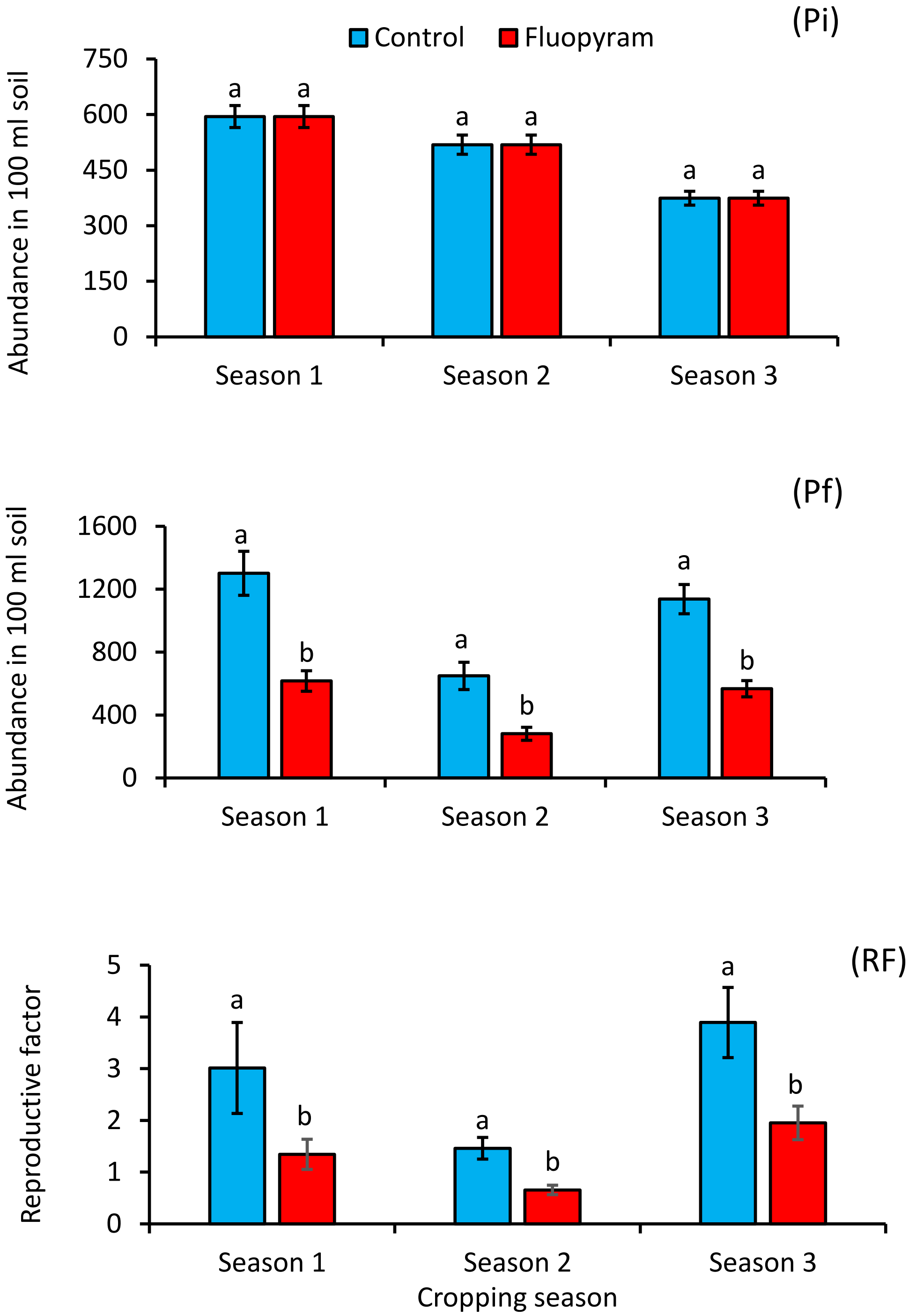

Figure 4.5 Effect of fluopyram-paper on initial (Pi), final (Pf), and reproductive factor (RF) of parasitic nematodes in potato cv. Shangi across three seasons in Nyandarua County.

Season 1 = long rains (Mar-May 2021); Season 2 = intermediate rains (Jul - Sep '21); Season 3 = short rains (Nov '21 - Jan '22). Bars sharing a letter within each season are not significantly different ($p < 0.05$).

Throughout the study, fourteen genera of PPN were identified, spanning eight distinct families. These included Aphelenchoididae (*Aphelenchoides* spp.), Aphelenchidae (*Aphelenchus* spp.), Belonolaimidae (*Tylenchorhynchus* spp.), Criconematidae (*Criconema* spp.), Heteroderidae (*Globodera* spp. and *Meloidogyne* spp.), Hoplolaimidae (*Hoplolaimus*, *Helicotylenchus*, and *Rotylenchus* spp.), Longidoridae (*Xiphinema* spp.), Pratylenchidae (*Pratylenchus* spp.), Trichodoridae (*Trichodorus* spp.), and Tylenchidae (*Filenchus* and *Tylenchus* spp.) (Fig. 4.6).

Among the economically important PPN, *Helicotylenchus* spp. and *Globodera* spp. were notably dominant, with their abundance significantly exceeding that of other genera (χ^2 = 413.39, $p < 0.001$). Furthermore, treatment effects were pronounced, as both *Helicotylenchus* spp. and *Globodera* spp. were consistently more abundant in control plots, with densities exceeding 115 nematodes per 100 ml of soil, compared to fluopyram-treated plots where densities remained below 57 nematodes per 100 ml of soil across all cropping seasons (χ^2 = 10.26, $p < 0.001$).

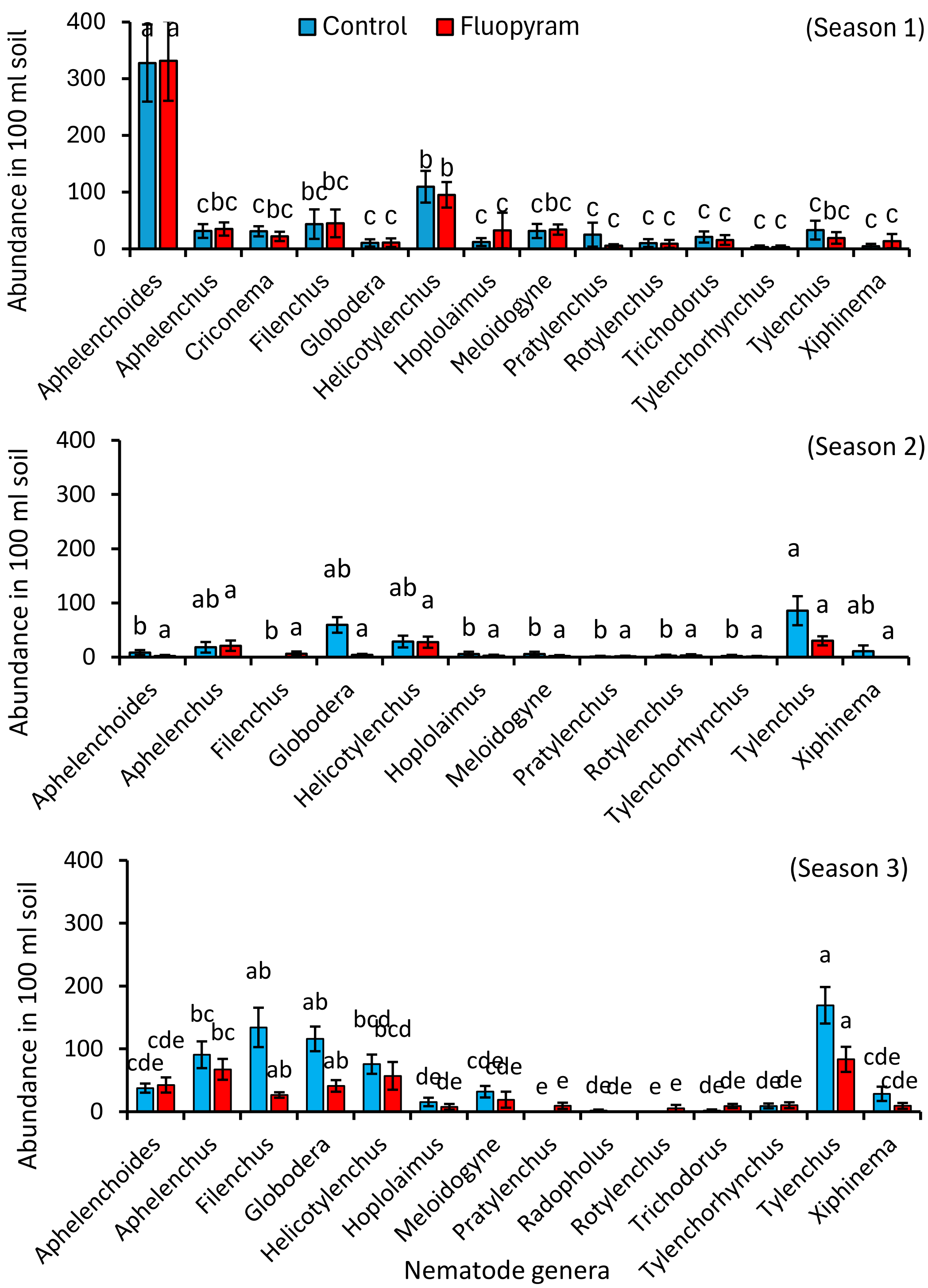

Figure 4.6 Effect of fluopyram-treated paper on nematode genera diversity in potatoes (cv. Shangi) in Nyandarua county, Kenya.

Season 1 = long rains (Mar - May '21); Season 2 = intermediate rains (Jul - Sep '21); Season 3 = short rains (Nov '21 - Jan '22). For each treatment, bars followed by the same letter are not significantly different at p < 0.05.

The use of fluopyram-treated banana paper resulted in a significant reduction in the number of cysts at the end of each cropping season ($\chi^2 = 19.33$, $p < 0.001$). This reduction corresponded with a significantly lower reproductive factor (RF) of cyst nematodes compared to the control ($\chi^2 = 9.53$, $p = 0.002$) (Fig. 4.7). In all seasons, plots treated with fluopyram-treated paper recorded up to a fourfold decrease in both cyst nematode counts and reproductive factor relative to the untreated control.

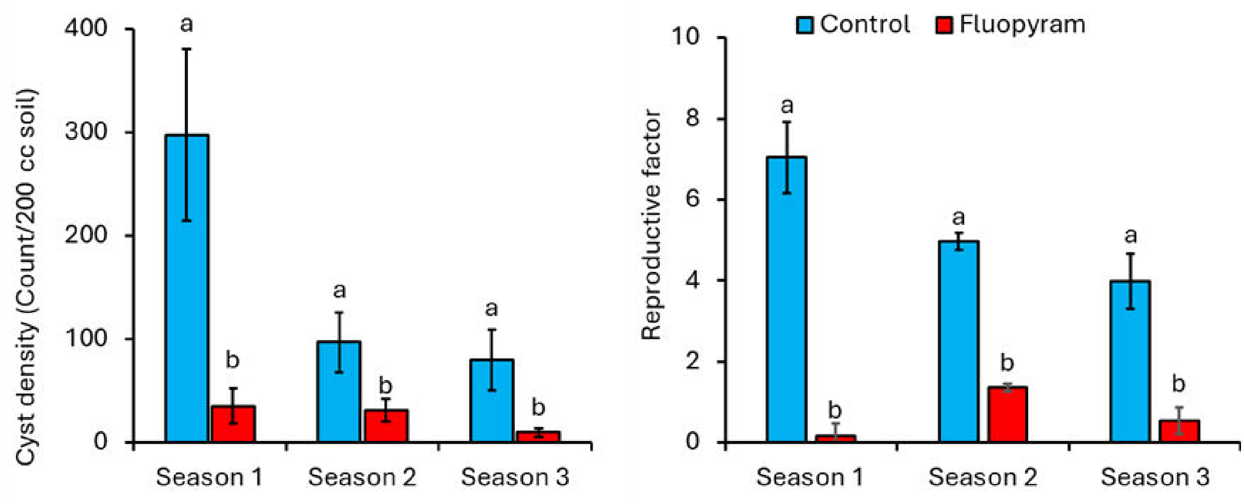

Figure 4.7 Effect of fluopyram-treated paper on the abundance of cysts and the cyst reproductive factor under potato (cv. Shangi) trials in Nyandarua county, Kenya.

Season 1 = long rains (Mar - May '21); Season 2 = intermediate rains (Jul - Sep '21); Season 3 = short rains (Nov '21 - Jan '22). Within each season, bars followed by the same letters are not significantly different at p < 0.05.

The total yield of potatoes was significantly ($\chi^2 = 14.57$, $p < 0.001$) higher in fluopyram-treated paper plots compared to control plots in season 2 (Appendix viii). However, the application of fluopyram-treated paper significantly increased the marketable yield of potatoes across all three growing ($\chi^2 = 15.63$, $p < 0.001$), as shown in Fig. 4.8. In contrast, there were no significant differences in the unmarketable yield between fluopyram-treated and control plots across the seasons.

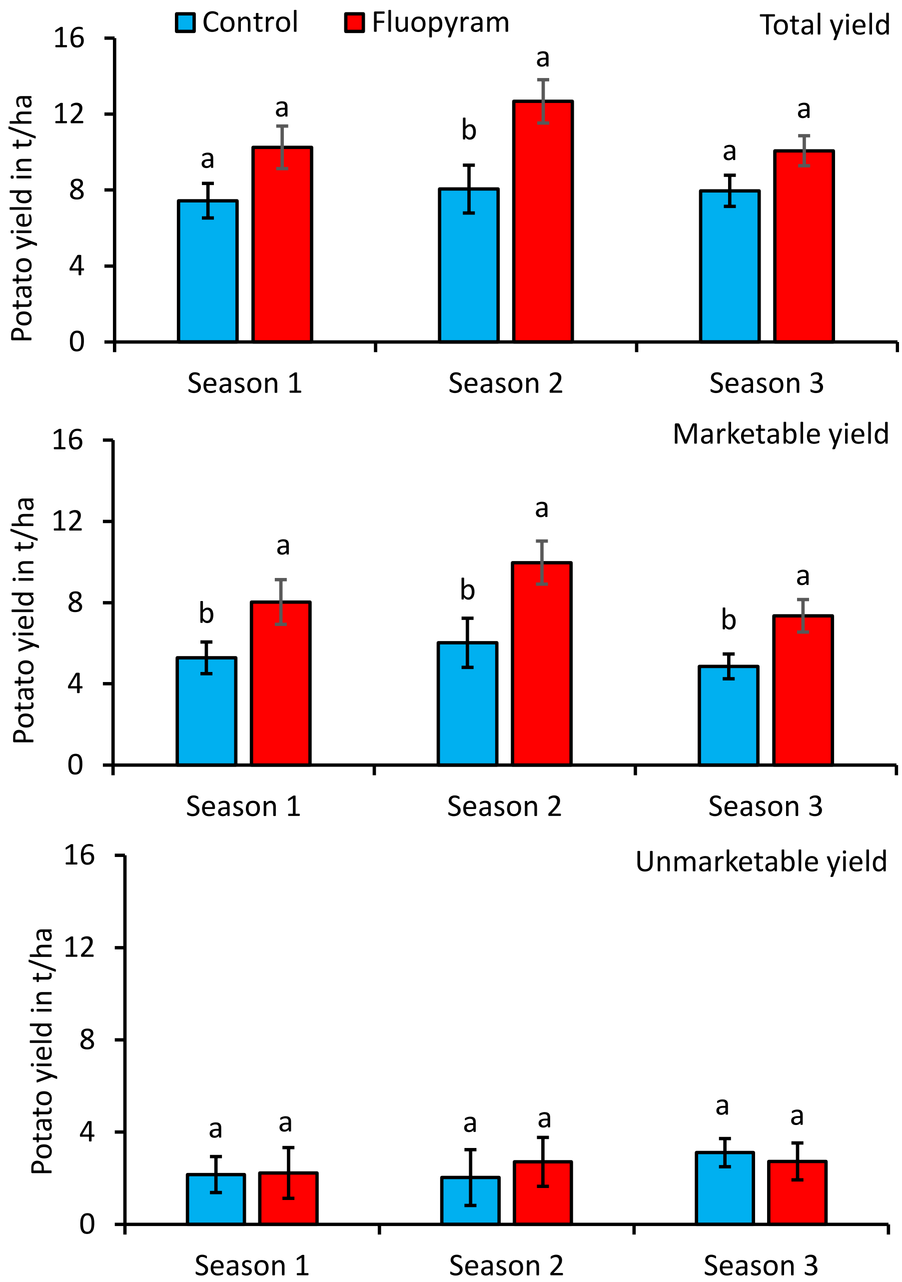

Figure 4.8 Effect of fluopyram-treated paper on the yield and quality of potatoes (cv. Shangi) in Nyandarua county, Kenya.

Season 1 = long rains (Mar - May '21); Season 2 = intermediate rains (Jul - Sep '21); Season 3 = short rains (Nov '21 - Jan '22). Within each season, bars followed by the same letter are not significantly different at $p < 0.05$.

4.4 Discussion

4.4.1 Effect of abamectin-and fluopyram-treated paper on nematodes

Abamectin-treated banana fibre paper led to significant reductions in PPN abundance, though its effects were more variable across seasons and cultivars. Abamectin, a macrocyclic lactone derived from *Streptomyces avermitilis*, binds to glutamate-gated chloride channels in nematodes, leading to neuromuscular paralysis and death (Sasanelli *et al.*, 2019). While its efficacy has been well documented under controlled conditions, field performance depends heavily on formulation, soil moisture, and delivery method.

In this study, abamectin-treated paper resulted in statistically significant suppression of PPN relative to untreated controls in one season but not the other. The inconsistency may be attributed to several factors such as the weather pattern, ultra-low dose used in the wraps, rapid photodegradation or microbial breakdown of abamectin in the soil, and spatial variability in nematode populations across replicates (Qiu *et al.*, 2022). Although abamectin's suppression was not consistent, its application through banana fibre paper still represents a promising alternative to broadcast applications, particularly when used in combination with other integrated strategies.

In the study, fluopyram-treated paper consistently led to significant reductions in both nematode abundance and their reproductive factor (RF), with some seasons recording over 60% suppression relative to the unwrapped control. Importantly, these reductions were more pronounced at final sampling (Pf) than at time of planting (Pi), indicating that fluopyram did not eliminate initial inoculum but rather suppressed population buildup during the crop cycle. This temporal dynamic corroborates earlier findings by Grabau and Chen (2016), who reported that fluopyram's field efficacy is primarily manifested in preventing second-stage juveniles (J2s) from establishing feeding sites rather than in immediate nematode mortality. The slow, sustained release of fluopyram from the banana fibre paper likely enhanced its persistence in the rhizosphere, maintaining effective concentrations over time without leaching, a key limitation of conventional soil drenching methods (Pirzada *et al.*, 2020).

4.4.2 Shifts in diversity of plant parasitic nematodes

The application of abamectin and fluopyram through banana fibre paper not only suppressed the overall abundance of PPN but also influenced the nematode community structure in the current study. Initial community profiles revealed a diverse assemblage of nematode taxa, including *Globodera*, *Meloidogyne* , *Helicotylenchus*, *Pratylenchus*, and *Tylenchorhynchus*, which are typically associated with intensively cropped systems (Moens *et al.*, 2009). Among these, *Helicotylenchus* and *Globodera* were the most abundant genera at the onset of the study, reflecting their adaptability and persistence in potato monocultures and the tendency of *Globodera* spp. to accumulate through continuous cropping (Turner and Subbotin, 2013).

4.4.3 Seasonal influences on nematode suppression

Suppression effects varied not only by treatment but also by cropping season. Seasonal variability in nematode suppression outcomes was observed in both trials. In wetter seasons, particularly long-rainy seasons, nematode reproduction was generally higher in the control plots, likely due to increased juvenile mobility and enhanced hatching rates (Marquez and Hajihassani, 2024). The banana fibre paper gradually degrades over time, with decomposition occurring more rapidly under wet conditions (Kamau *et al*., 2024), facilitating sustained release of the active ingredient. This mechanism likely contributed to the enhanced nematode suppression observed during long rainy seasons. Suppression in fluopyram-treated plots remained relatively stable across seasons, suggesting resilience of the delivery mechanism to environmental fluctuations. These observations affirm the findings of Jones *et al.* (2013), who emphasized the importance of deploying nematicides in formulations that buffer against climatic variability in smallholder agroecosystems.

4.4.4 Effect of treated banana paper on potato yield increment

Abamectin paper resulted in high yield gains relative to the control. In some seasons and cultivars, especially where initial nematode pressure was high, abamectin plots showed up to 30% increases in total yield. However, the differences were more pronounced in Sherekea than in Shangi. This suggests a possible synergistic effect where resistance complemented abamectin's nematicidal activity, reducing nematode pressure below critical damage thresholds (Sasanelli *et al*., 2019). Abamectin's yield impact may also be influenced by its relatively short half-life and lower mobility in soil. Unlike other nematicides which remain active for extended periods in the rhizosphere, abamectin can degrade rapidly, particularly in warm or biologically active soils (Feng *et al.*, 2023). The banana paper carrier may mitigate this by slowing release (Pirzada *et al.*, 2023), but field persistence remains a limiting factor for season-long nematode control. As a result, abamectin-treated plots may experience early season protection, with diminishing effect over time (Kamau *et al.* 2024).

Fluopyram-treated banana paper consistently yielded higher tuber output than control across all seasons. In some trials, fluopyram-treated plots recorded yield increases of 30–60% compared to the control, echoing findings from previous field studies where fluopyram applications led to significant yield improvements in nematode-infested fields (Grabau and Chen, 2016; Desaeger *et al.*, 2020). These findings could be attributed to several factors. By suppressing sedentary endoparasitic nematode species like *Globodera* spp. and *Meloidogyne* spp., fluopyram preserves root system integrity, which in turn supports greater water and nutrient uptake (Hagan *et al*., 2024). This translates to better canopy development, higher photosynthetic rates, and improved tuber filling (Saleh *et al*. 2022). These physiological benefits have been confirmed in studies using SDHI-based nematicides where root mass and function were significantly improved relative to untreated controls (Hungenberg *et al*., 2016).

CHAPTER FIVE: EFFICACY OF NEMATICIDE APPLICATION AND HOST RESPONSE TO NEMATODE SUPPRESSION AND YIELD PERFORMANCE IN POTATOES

5.1 Introduction

The selection of potato cultivars with varying nematode resistance levels is a key component of integrated nematode management. Resistant cultivars, especially those containing the H1 gene, have been demonstrated to suppress *Globodera rostochiensis* and sometimes *G. pallida* by preventing complete nematode development and reproduction within the host roots, thereby reducing population buildup in the field (Spychalla and De Jong, 2024; Onditi and Whitworth, 2024). In Kenya, studies have identified cultivars such as Nyota that show partial resistance to PCN under greenhouse conditions (Ochieng *et al*., 2024). This resistance translated into lower reproductive indices and reduced root damage compared to susceptible cultivars like Desiree, indicating the practical importance of genetic resistance in smallholder systems (Ochieng, 2023).

However, resistance alone often falls short under high nematode pressure or when faced with mixed nematode species. Field trials in Nyandarua County showed that nematode management outcomes differed significantly between susceptible and resistant cultivars treated with fluopyram (Mbiyu *et al*., 2022). While fluopyram reduced cyst densities in both cultivar types, the impact on yield and nematode suppression was more pronounced in susceptible cultivars, underscoring treatment by cultivar interactions (Li *et al*., 2021). Thus, combining host resistance with chemical interventions such as fluopyram or abamectin can enhance nematode control efficacy, particularly where resistance alone is inadequate.

Fluopyram and abamectin exhibit different modes of action and ecological profiles in the suppression of PPN. Abamectin functions as a neurotoxic biological agent that causes irreversible paralysis in nematodes, with efficacy demonstrated against *Globodera pallida* at moderate concentrations (LD_{50} ≈ 14.4 µg/mL) in both in vitro and pot trials, performing comparably to conventional chemical nematicides (Sasanelli *et al*., 2019). In contrast, fluopyram, an SDHI fungicide with nematicidal activity, demonstrates broad-spectrum and more persistent suppression of plant parasitic nematode motility and hatching. It inhibits juvenile mobility in *Meloidogyne* and *Rotylenchulus* spp. at concentrations as low as 1–2 µg/mL, though its effects may be nematostatic and reversible depending on dose and species (Faske and Hurd, 2015). Field studies on cyst nematodes like *Heterodera glycines* confirm significant reductions in final nematode populations but seldom achieve complete population decline (Roth *et al*., 2020). This study compared the effect of chemical nematicide against chemically treated banana fibre paper on the suppression of PPN and its potential to enhance yield.

5.2 Materials and Methods

5.2.1 Experimental design

A Type I experimental design (designed and managed by the researcher) was established. A split-plot design was used at an on-farm site in Engineer, Kinangop, Nyandarua (coordinates: -0.72786° S, 36.6542° E). The main plot factor was potato cultivar, while the subplot factor was banana fibre paper treatment. Two potato cultivars with varying resistance levels were compared: Shangi (susceptible to nematodes) and Manitou (resistant to nematodes) (Mburu *et al*., 2020).

For the treatments, three banana paper treatments: abamectin-treated paper, fluopyram-treated paper and untreated fibre paper were assessed. Direct application of the nematicide Velum Prime (400 g/L fluopyram), applied at a standard rate of 1.25 L/ha, was included as a conventional nematicide treatment. All treatments were compared to an absolute control where potato seed tubers remained unwrapped and untreated as is farmers' practice. Each plot measured 3 x 4 m, with 50 seed tubers planted per plot at a spacing of 30 cm between plants and 75 cm between rows totaling to 41,666 plants per hectare. The treatments were replicated three times in a randomized complete block design (RCBD). The experiment was repeated over two consecutive cropping seasons during the long rain and short rain season (Table 5.1)

Table 5.1 Rainfall and temperature during the study period

Season	Rain	Period	Rainfall (mm)	Mean temperature
Season 1	Long rains	Mar - May '22	428.15	18.02˚C
Season 2	Intermediate rains	Jul - Sep '22	437.12	15.79˚C

Seasonal rainfall collected during the study period
Temperature recorded daily and average between lowest and highest temperature

Good agronomic practices were observed throughout the trial period. Di-ammonium phosphate (DAP) fertilizer was applied to each plot at the time of planting at a rate of 50 kg/acre. As a top dress fertilizer, calcium ammonium nitrate (CAN) was added once all plots had achieved >80% germination rate. Weeding was performed twice in each season at 30- and 60-days post planting to remove unwanted plants. The fungicides Ridomil gold (Metalaxyl-M 40 g/Kg + Mancozeb 640 g/Kg) at rate of 2.5 kg/ha and Revus (Mandipropamid 250 g/L) at a rate of 0.6 L/ha were administered interchangeably to control late blight of potatoes among other diseases. In the first season, the trials were strictly rainfed while in the second season, irrigation was evenly performed on the plots upon delayed germination.

5.2.2 Soil sampling and data collection

Soil samples for nematode analyses, nematode assessment and yield data followed the description in methodology section 3.3 – 3.4.

5.2.3 Data analyses

Data were tested for normality and homogeneity of variance using the Shapiro–Wilk test and Levene's test, respectively, to ensure that assumptions of parametric analysis were met. Where necessary, nematode count data were log-transformed [$\log_n(x + 1)$] to stabilize variances and normalize distribution. Three-way analysis of variance (ANOVA) was used to evaluate the main and interaction effects of potato cultivar, banana fibre paper treatment, and cropping season on PPN density, cyst counts, nematode diversity and crop yield. Where no significant seasonal effects were detected, data were pooled across seasons. Post-hoc comparisons were performed using Tukey's Honest Significant Difference (HSD) test at a 95% confidence level ($p < 0.05$). In cases where interaction effects were significant, pairwise comparisons were further explored to interpret treatment-specific effects. All statistical analyses were conducted using R version 4.2.3 (R Core Team, 2023). Results were reported as mean ± standard error, and graphical outputs were generated using the ggplot2 package (Wickham, 2009).

5.3 Results

5.3.1 Prevalent plant parasitic nematodes in the study

Morphological analysis of the parasitic nematodes showed that there were twelve nematode genera recovered from the study. Nematode community composition shifted across treatments, with some genera showing treatment-specific suppression between the two cropping seasons. In Season 1, treatment significantly influenced nematode abundance ($\chi^2 = 10.51$, $p = 0.033$), while genus varied significantly ($\chi^2 = 169.07$, $p < 0.001$) (Table 5.1). The main effect of cultivar was not significant ($\chi^2 = 0.47$, $p = 0.49$) and therefore data were pooled together (Appendix viii). There was a significant treatment by genus interaction ($\chi^2 = 71.81$, $p = 0.005$), indicating that responses varied by nematode genus. A significant treatment × cultivar × genus three-way interaction was also observed ($\chi^2 = 63.87$, $p = 0.027$). However, interactions between treatment and cultivar ($\chi^2 = 2.87$, $p = 0.579$), and between cultivar and genus ($\chi^2 = 12.43$, $p = 0.33$), were not significant. Population densities of *Globodera* spp. were notably high under fluopyram paper (85.67) and the control (50.67) (Table 5.1). Conversely, *Helicotylenchus* spp., *Hoplolaimus* spp., and *Meloidogyne* spp. recorded low numbers across all treatments. *Trichodorus* spp. was most abundant under fluopyram treatment (47.67), while *Criconema* spp. showed a marked decline in the fluopyram and control plots.

In Season 2, genus again varied significantly ($\chi^2 = 189.93$, $p < 0.001$), but in contrast, treatment did not have a significant effect ($\chi^2 = 7.19$, $p = 0.12$). None of the interactions were significant, including treatment × cultivar ($\chi^2 = 2.18$, $p = 0.70$), treatment × genus ($\chi^2 = 50.19$, $p = 0.24$), cultivar × genus ($\chi^2 = 11.31$, $p = 0.41$), or the three-way interaction of treatment × cultivar × genus ($\chi^2 = 29.52$, $p = 0.95$) (Table 5.1).

Table 5.2 Nematode community composition under banana fibre paper treatments across two cropping seasons in Nyandarua county, Kenya

	Genus	Control	Abamectin-paper	Direct fluopyram	Fluopyram-paper	Untreated paper
Season 1	*Aphelenchus*	21 ± 5 b B	27 ± 8 b AB	13 ± 4 bc B	51 ± 18 a A	21 ± 9 b B
	Criconema	2 ± 2 c B	16 ± 9 b A	0 ± 0 e B	4 ± 4 cd B	2 ± 2 c B
	Filenchus	23 ± 7 b AB	54 ± 32 a A	8 ± 4 bcd B	48 ± 11 a A	52 ± 21 a A
	Globodera	57 ± 22 a AB	20 ± 10 b B	10 ± 4 bc B	86 ± 29 a A	51 ± 22 a AB
	Helicotylenchus	0 ± 0 c A	1 ± 1 c A	0 ± 0 e A	4 ± 4 cd A	0 ± 0 c A
	Hoplolaimus	0 ± 0 c A	0 ± 0 c A	2 ± 2 de A	3 ± 3 cd A	0 ± 0 c A
	Meloidogyne	2 ± 2 c A	3 ± 3 c A	0 ± 0 e A	0 ± 0 d A	2 ± 2 c A
	Trichodorus	11 ± 5 b B	14 ± 5 b B	48 ± 19 a A	25 ± 8 b AB	30 ± 17 ab AB
	Xiphinema	0 ± 0 c A	0 ± 0 c A	3 ± 3 de A	3 ± 3 cd A	3 ± 3 c A
Season 2	*Aphelenchoides*	1062 ± 219 a A	900 ± 378 ab A	912 ± 373 a A	1666 ± 493 a A	967 ± 324 a A
	Aphelenchus	56 ± 20 d B	154 ± 50 d A	144 ± 34 bc A	191 ± 54 c A	184 ± 77 de A
	Criconema	0 ± 0 f A	0 ± 0 f A	5 ± 5 f A	0 ± 0 e A	0 ± 0 f A
	Filenchus	23 ± 15 de B	121 ± 46 d AB	81 ± 81 cdef AB	260 ± 121 c A	133 ± 74 e AB
	Globodera	400 ± 123 c A	211 ± 125 cd AB	56 ± 39 de B	273 ± 178 c AB	460 ± 137 bc A
	Helicotylenchus	496 ± 335 bc BC	1233 ± 350 a A	6 ± 6 f C	911 ± 376 ab AB	931 ± 375 ab AB
	Hoplolaimus	0 ± 0 f A	0 ± 0 f A	0 ± 0 f A	14 ± 14 e A	0 ± 0 f A
	Meloidogyne	44 ± 31 d A	42 ± 19 e A	18 ± 18 ef A	10 ± 10 e A	305 ± 291 bcde A
	Rotylenchus	0 ± 0 f B	0 ± 0 f B	56 ± 43 de A	0 ± 0 e B	0 ± 0 f B
	Trichodorus	594 ± 66 b B	695 ± 168 b B	1073 ± 119 a A	701 ± 106 b B	564 ± 124 ab B
	Tylenchus	440 ± 221 bc B	389 ± 121 c B	323 ± 153 b B	1005 ± 285 ab A	199 ± 49 de B
	Xiphinema	6 ± 6 ef C	34 ± 6 e C	119 ± 64 bcd B	67 ± 27 d BC	327 ± 91 cd A

[1]Control = farmer's practice with no banana paper or treatment applied

Means followed by the same lowercase letter(s) along the treatment columns in each season are not statistically different at $p < 0.05$

For each nematode genera within each season, means followed by the same uppercase letter(s) across the treatments are not statistically different at $P < 0.05$

Season 1 is long rains from March to May and season 2 is intermediate rains from July to September 2022

5.3.2 Effect of banana paper treatments on nematode densities

The influence of treatment, potato cultivar, and their interaction on plant parasitic nematode (PPN) density was assessed separately for each cropping season. In Season 1, treatment had a significant effect on PPN density ($\chi^2 = 337.23$, $p < 0.001$), whereas cultivar ($\chi^2 = 2.03$, $p = 0.15$) and the treatment × cultivar interaction ($\chi^2 = 4.58$, $p = 0.33$) were not significant. For cv. Shangi, all treated plots recorded lower PPN densities than the control as depicted in Table 5.2. Direct fluopyram produced the highest suppression, lowering nematode numbers by approximately 77% relative to the control. Fluopyram paper and abamectin paper each reduced populations by about 33% and 27%, respectively, while untreated banana paper lowered counts by roughly 28%. For cv. Manitou, a similar pattern was observed. Fluopyram paper and direct fluopyram achieved reductions of around 60% and 58%, respectively, relative to the control. Abamectin paper resulted in a nematode decline of up to 46%, while untreated paper lowered nematode numbers by 40% when compared to the control plots (Table 5.2).

In Season 2, all main factors and their interaction significantly affected PPN density (cultivar: $\chi^2 = 89.85$, $p < 0.001$; treatment: $\chi^2 = 64.26$, $p < 0.001$; interaction: $\chi^2 = 60.40$, $p < 0.001$). Within cv. Shangi, all treatments supressed PPN densities relative to the control (Table 5.2). Direct fluopyram achieved the strongest reduction of approximately 71%, followed closely by fluopyram paper at 67%. Abamectin paper resulted in a 29% reduction, and untreated banana paper lowered nematode counts by about 48%. For cv. Manitou, treatment responses were more variable but still significant at $p \leq 0.05$. Only fluopyram-based treatments reduced PPN densities by at least 18% relative to the control (Table 5.2).

Table 5.3 Effect of banana fibre paper treatments and chemical controls on plant parasitic nematode density in potatoes (cvs. Shangi and Manitou) in Nyandarua County, Kenya.

	Season 1		Season 2	
Treatment	Shangi	Manitou	Shangi	Manitou
Abamectin paper	167.67 ± 82.39 b A	101.33 ± 11.84 bc A	5269.35 ± 885.37 b A	1863.95 ± 365.79 ab B
Control	228.33 ± 65.53 a A	189.33 ± 34.11 a A	7381.15 ± 232.87 a A	1956.09 ± 227.79 ab B
Direct fluopyram	51.67 ± 18 c A	80.33 ± 2.73 cd A	2104.7 ± 494.19 d A	1611.3 ± 293.14 b A
Fluopyram paper	152.67 ± 38.33 b A	76.33 ± 9.74 d A	2445.56 ± 499.04 cd A	1232.7 ± 94.26 c B
Untreated paper	164.33 ± 40.17 b A	113 ± 16.33 b A	3852.56 ± 424.51 c A	2234.97 ± 222.97 a B

Mean ± SE represents nematode abundance represented per 100 ml of soil.
Season 1 is long rains from Mar - May '22, season 2 is Intermediate rains from Jul - Sep '22.
Means followed by the same lowercase letters along the treatment columns are not statistically different at $p < 0.05$
Means followed by the same uppercase letters across the cultivars in each season are not statistically different at $p < 0.05$

5.3.3 Effect of banana paper treatments on cyst nematode multiplication

The influence of treatment, potato cultivar, and their interaction on the nematode reproductive factor was assessed across two cropping seasons. Treatment had a highly significant effect on reproductive factor ($\chi^2 = 27.64$, $p < 0.001$) across the potato cultivars. In cv. Manitou, during the first season, only direct fluopyram application significantly reduced the nematode reproductive factor, whereas in the second season, all treatments produced significantly lower reproduction compared to the untreated control (Fig. 5.1). On the other hand, in cv. Shangi, fluopyram-based treatments and abamectin paper recorded the lowest reproductive factors relative to the control across seasons.

The main effect of cultivar was also significant ($\chi^2 = 4.25$, $p = 0.03$), with cv. Shangi exhibiting higher reproductive factors than cv. Manitou, particularly in the first season. However, the interaction between treatment and cultivar was not statistically significant ($\chi^2 = 1.95$, $p = 0.71$).

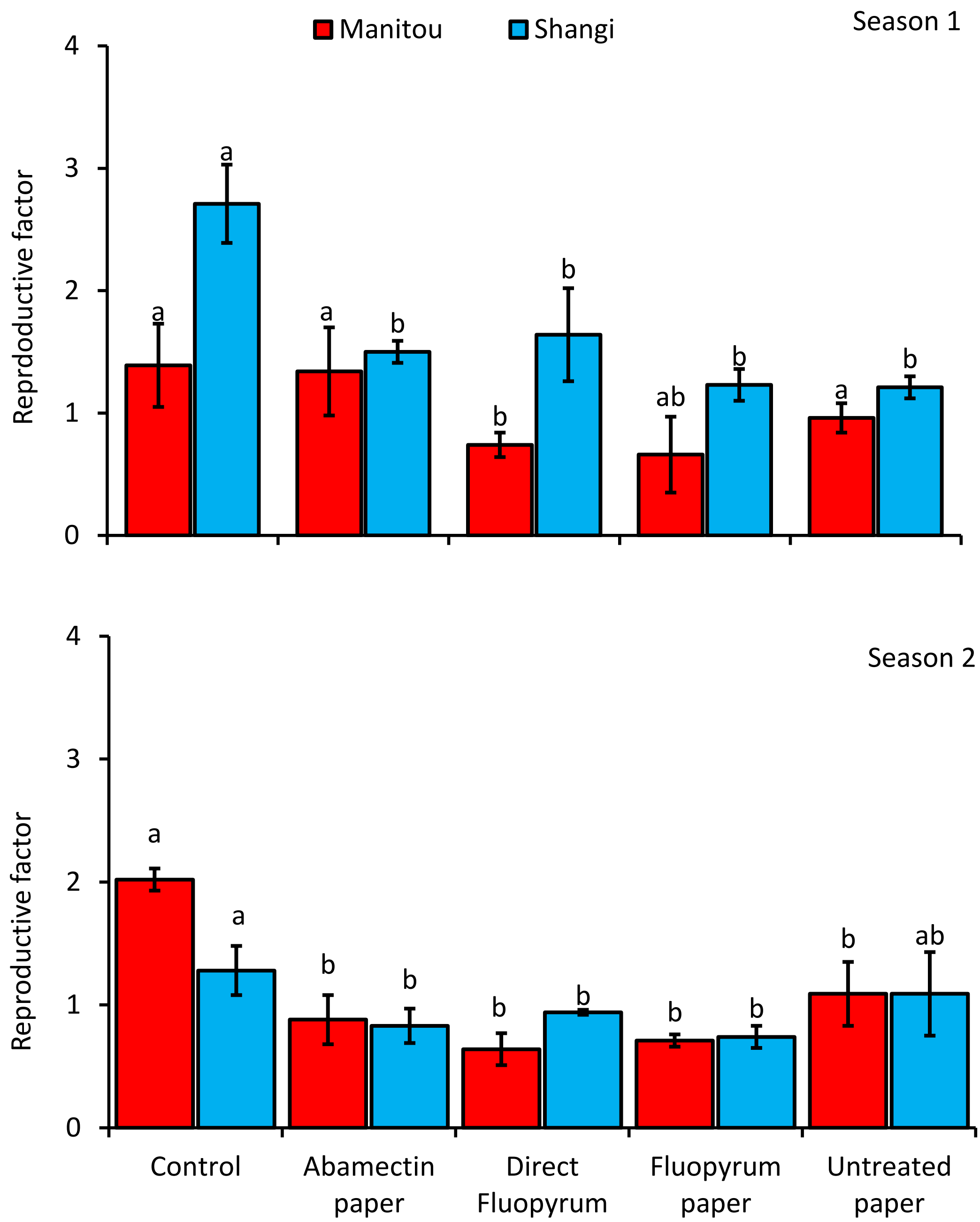

Figure 5.1 Reproductive factor of potato cyst nematodes in two potato cultivars (cvs. Shangi and Manitou) under banana fibre treatments across two seasons in Nyandarua County, Kenya.

Season 1 is long rains from Mar - May '22, season 2 is Intermediate rains from Jul - Sep '22. Within each potato cultivar, bars followed by the same letter are not significantly different at $p < 0.05$.

5.3.4 Yield of potatoes under banana paper treatments

Potato yield varied significantly across treatments, cultivars, and seasons (χ^2 = 498.58, p < 0.001; χ^2 = 134.92, p < 0.001; χ^2 = 85.38, p < 0.001, respectively). A significant three-way interaction between cultivar, treatment, and season (χ^2 = 9.76, p = 0.045) indicated that the response to treatments differed between cultivars and seasons (Fig. 5.2). While cultivar by season interactions were also significant (χ^2 = 10.12, p = 0.001), the interactions of cultivar by treatment (χ^2 = 5.42, p = 0.25) and treatment by season (χ^2 = 7.25, p = 0.12) were not statistically significant.

In season 1 under potato cultivar Shangi, the highest yield was observed under direct fluopyram application (9.1 t/ha), followed by fluopyram paper (3.8 t/ha), abamectin paper (2.1 t/ha), untreated paper (1.7 t/ha), and control (1.3 t/ha). A similar trend was noted in the second season, where direct fluopyram again led with a mean yield of 10.8 t/ha, followed by fluopyram-treated paper (8.3 t/ha), abamectin paper (7.1 t/ha), untreated paper (3.4 t/ha), and control (2.9 t/ha) (Fig. 5.2).

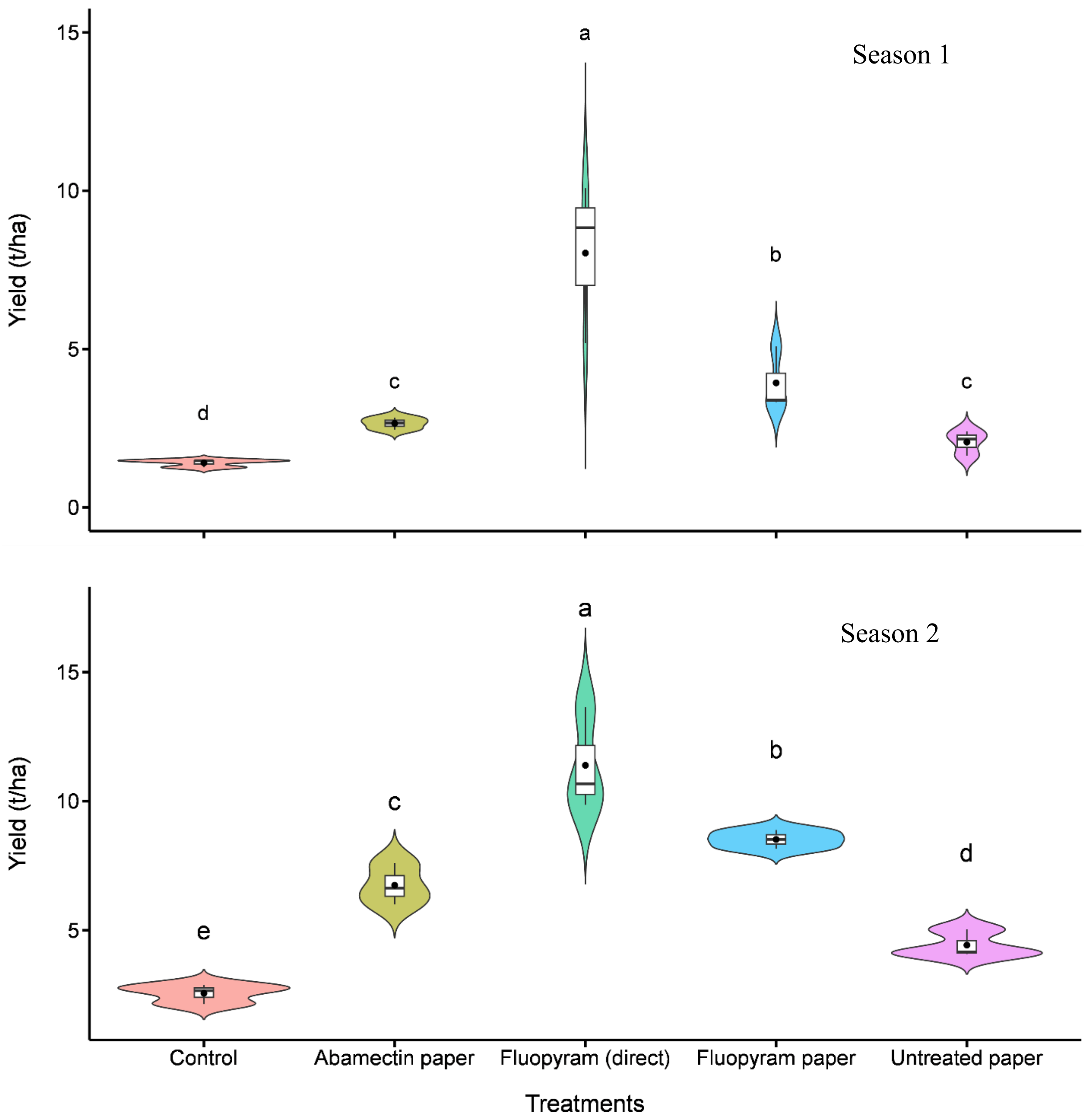

Figure 5.2 Violin plots showing the yield response of potato cv. Shangi to banana paper treatments in Nyandarua county, Kenya.

Season 1 is long rains from Mar - May '22, season 2 is Intermediate rains from Jul - Sep '22. Violins followed by the same letters are not significantly different at $p < 0.05$.

For Manitou, treatment effects were also evident. In Season 1, direct fluopyram produced the highest mean yield (11.2 t/ha), followed by fluopyram paper (9.8 t/ha), abamectin paper (7.1 t/ha), untreated paper (5.2 t/ha), and control (3.9 t/ha). In Season 2, direct fluopyram remained the top performer (13.6 t/ha), followed by fluopyram paper (10.5 t/ha), abamectin paper (7.2 t/ha), untreated paper (6.1 t/ha), and control (4.6 t/ha) (Fig. 5.3).

Across both cultivars and seasons, fluopyram consistently resulted in the highest yields, while the control and untreated paper had the lowest. Abamectin paper showed moderate effectiveness, with higher yields than untreated controls but lower than fluopyram-based treatments. Notably, yield responses in Shangi were generally lower than in Manitou under all treatments except direct fluopyram application.

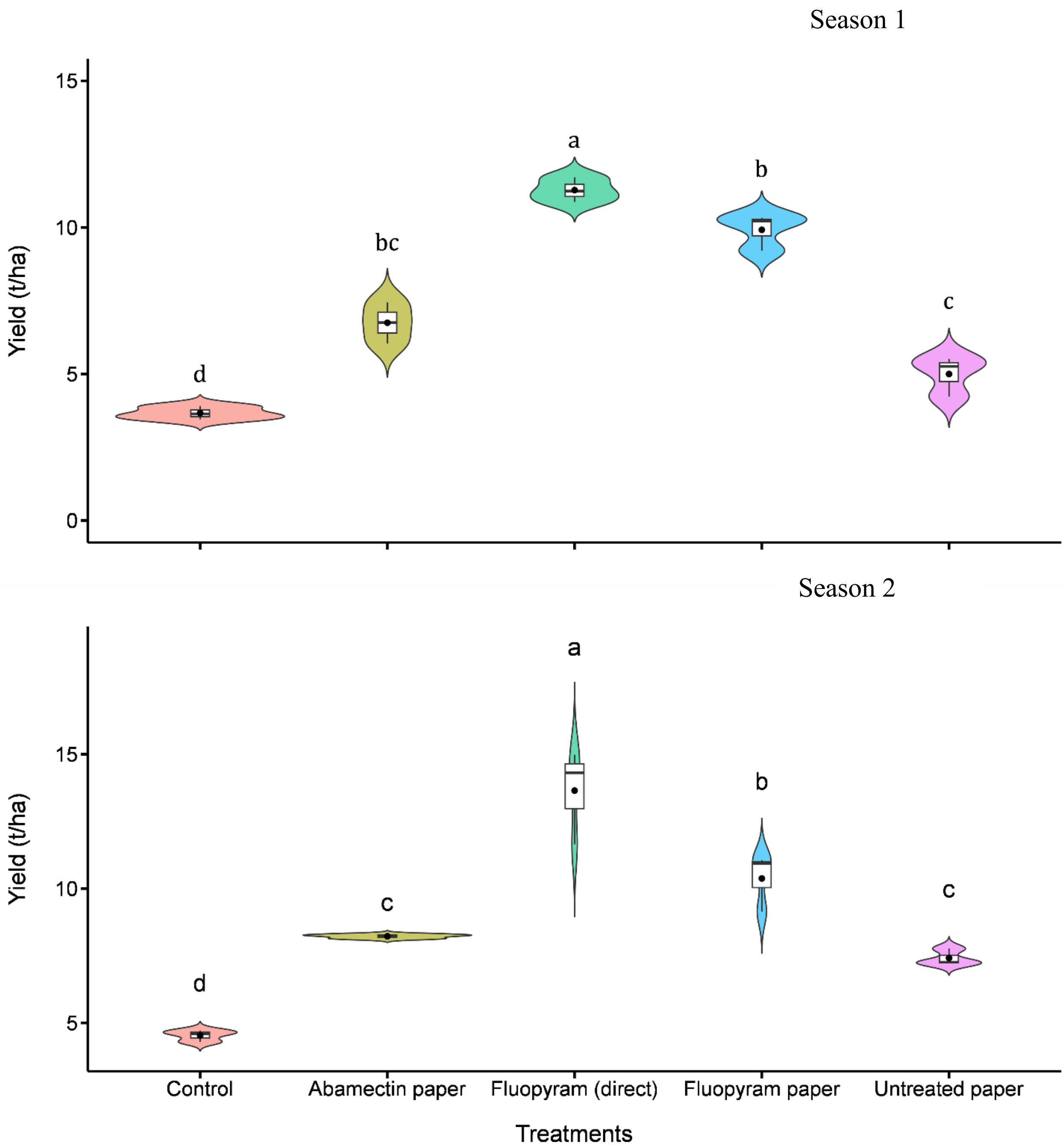

Figure 5.3 Violin plots showing the yield response of potato cv. Manitou to banana paper treatments in Nyandarua county, Kenya.

Season 1 is long rains from Mar - May '22, season 2 is Intermediate rains from Jul - Sep '22. Violins followed by the same letters are not significantly different at $p < 0.05$

5.4 Discussion

The findings from this study underscore the central role of host plant resistance in managing plant parasitic nematodes (PPN) in smallholder potato production systems. Across multiple seasons, the resistant cultivar Manitou consistently exhibited lower nematode reproductive factors compared to the susceptible cultivar Shangi, even in untreated plots. These outcomes are in agreement with previous studies showing that resistance genes such as H1 disrupt the life cycle of *Globodera* spp. in potato roots, limiting cyst formation and associated yield losses (Otieno *et al*., 2023; Spychalla and De Jong, 2024). Such genetic resistance mechanisms appear to prevent the establishment and multiplication of juveniles, thereby reducing nematode reproductive success over successive cropping cycles. Similar patterns have been reported in other Kenyan potato cultivars, such as Nyota, which demonstrate partial but stable resistance under both greenhouse and field conditions (Ochieng *et al*., 2024). These observations reinforce the notion that cultivar choice is a critical first line of defence against PPN. Similar findings from other trials further confirm that resistant cultivars consistently maintain lower PPN densities than susceptible lines, even under variable environmental and pest pressures (Tylka *et al*., 2019; Mbiyu *et al*., 2022), highlighting the broad applicability of genetic resistance across diverse agroecological conditions.

Nematode densities varied across treatments and cultivars, further illustrating the importance of targeted chemical interventions in complementing host resistance. Direct application of fluopyram consistently produced the lowest total nematode counts across both resistant and susceptible cultivars, corroborating its documented ability to inhibit succinate dehydrogenase, thereby impeding juvenile motility and egg hatching even at low concentrations (Zhang *et al*., 2014; Faske and Hurd, 2015; Jorgensen *et al*., 2024). Fluopyram-treated plots consistently outperformed banana fibre paper treatments, suggesting that while controlled-release systems offer sustained activity, direct applications ensure immediate and peak nematicidal effects.

These observations are consistent with findings in potato and soybean systems, where direct succinate dehydrogenase inhibitor (SDHI) applications provided superior suppression compared to slow-release or matrix-based formulations, particularly in high-pressure nematode environments (Beeman and Tylka, 2018; Roth *et al*., 2020). The consistency of fluopyram efficacy across multiple seasons and cultivars in this study reinforces the value of SDHI nematicides as reliable tools for integrated nematode management.

Banana fibre paper-based delivery systems also demonstrated strong nematode suppression, although slightly less effective than direct chemical application. Fluopyram-loaded paper achieved the second-highest reductions in nematode densities, indicating that the controlled-release properties of the biodegradable matrix sustained the activity of the active ingredient across cropping cycles. This pattern aligns with previous studies showing that biodegradable matrices, including paper-based carriers, can maintain nematicide bioavailability and controlled release, although peak suppression may occur slightly later than with direct application (Sasanelli *et al*., 2021; Ochola *et al*., 2022). Abamectin-treated paper also reduced nematode densities significantly compared to untreated controls, particularly under moderate to high nematode pressure, supporting prior studies demonstrating its reliability as a neurotoxic nematicide. Although slower-acting than SDHI compounds, abamectin effectively disrupts neural transmission in nematodes, providing sustained suppression over time (Sasanelli *et al*., 2019).

The untreated banana-fibre paper, even without added nematicide, also contributed to nematode suppression and yield improvement. In the field trials it achieved moderate reductions in PPN densities compared to absolute controls, likely by affecting juvenile movement or root-exudate interactions as reported in earlier banana-paper wrap technologies (Ochola *et al*., 2022). This supports findings that biodegradable ligno-cellulosic wraps reduce nematode penetration and can enhance yield under smallholder conditions (Pirzada *et al*., 2023; Kamau *et al*., 2024). Although its efficacy was lower than nematicide-loaded papers, its performance suggests value as a low-input option or as part of integrated strategies

Cyst multiplication results reinforced the importance of integrating host plant resistance with chemical interventions. Manitou plots treated with direct fluopyram consistently produced the lowest cyst counts, highlighting the synergistic effect of combining genetic resistance with chemical reinforcement. This observation aligns with integrated nematode management frameworks, which advocate for stacking host plant resistance with selective chemical tools to enhance control efficacy, particularly under high pest pressure (Tylka *et al*., 2019; Li *et al*., 2021). Susceptible Shangi benefited more markedly from chemical interventions, with fluopyram significantly reducing cyst counts relative to untreated or paper-treated plots, consistent with reports that susceptible cultivars often require targeted chemical inputs to achieve effective nematode suppression (Beeman and Tylka, 2018; Li *et al*., 2021). These results underscore the value of a combined approach, wherein resistant cultivars provide baseline control and chemical interventions compensate for susceptible genotypes under elevated nematode infestation.

Yield outcomes closely mirrored patterns of nematode suppression, reinforcing the direct relationship between effective PPN management and agronomic performance. Direct fluopyram application consistently produced the highest tuber yields across both cultivars and seasons, followed by fluopyram paper, then abamectin paper, with untreated plots yielding the lowest. These findings confirm the tight link between nematode management and crop productivity, as observed in multiple field trials in Europe and North America, where fluopyram-treated plots achieved 30–40% higher yields than untreated controls under high nematode pressure (Beeman and Tylka, 2018; Roth *et al*., 2020). The resistant cultivar Manitou maintained higher yields than Shangi in untreated plots, reflecting intrinsic genetic resistance and naturally lower nematode reproduction (Otieno *et al*., 2023; Spychalla and De Jong, 2024).

Chemical interventions, particularly direct fluopyram, effectively compensated for susceptibility in Shangi, with treated yields approaching those of treated Manitou, demonstrating the practical value of integrating chemical and genetic strategies. Abamectin-treated paper provided intermediate yield gains, particularly under higher nematode pressure, consistent with previous evidence that slower-acting nematicides can still enhance yields if applied appropriately (Sasanelli *et al*., 2019). These results collectively support the integration of host plant resistance with either direct or paper-based nematicide delivery to optimize both nematode suppression and crop productivity, in line with established integrated nematode management principles (Sikora and Roberts, 2018). Similarly, untreated banana-fibre paper contributed to modest yield improvements over absolute controls. Plots with untreated paper yielded slightly higher tubers than unwrapped controls, reflecting its ability to provide physical protection to seed pieces, moderate moisture retention, and possibly enhanced root development (Ochola *et al*., 2022; Pirzada *et al*., 2023; Kamau *et al*., 2024). While these gains were less than those achieved with nematicide-loaded papers, the untreated paper demonstrates potential as a low-input, sustainable intervention to support smallholder potato productivity, particularly in systems where chemical inputs are limited

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CHAPTER SIX: EFFECTS OF CHEMICALLY AND BIOLOGICALLY TREATED BANANA FIBRE PAPER ON PARASITIC NEMATODE COMMUNITIES IN POTATO AND PEA SYSTEMS

6.1 Introduction

Plant parasitic nematodes (PPN) are among the most damaging soilborne pests in smallholder agroecosystems, with particularly devastating effects on crops like potato (*Solanum tuberosum*) and pea (*Pisum sativum*) that are often grown in rotation. In Kenya and much of sub-Saharan Africa, *Globodera* spp. (potato cyst nematodes) and *Meloidogyne* spp. (root-knot nematodes) cause extensive yield losses, compounded by limited access to resistant cultivars and effective nematode management strategies (Desaeger *et al*., 2020; Ndinya *et al*., 2020; Salgado *et al*., 2021). Conventional chemical nematicides such as fluopyram and abamectin have shown consistent efficacy in suppressing PPN populations (Faske and Hurd, 2015; Sasanelli *et al*., 2019), but their widespread use is increasingly constrained by ecological concerns such as soil, water and animal contamination, input costs, and regulatory restrictions. These challenges underscore the urgent need for sustainable, biologically based nematode control strategies that are both effective and economically viable for smallholder contexts (Coyne *et al*., 2018a).

For biological control of nematodes, *Trichoderma* spp. is arguably one of the most important rhizosphere fungi due to its bilateral role in plant protection and soil health enhancement (Afridi *et al*., 2022). Numerous studies have confirmed its efficacy in suppressing PPN across diverse crops (Kiriga *et al*., 2018). The genus exhibits multiple biocontrol mechanisms, including antibiosis, enzymatic degradation (through chitinases and proteases), competition for space and nutrients, direct mycoparasitism, and the production of nematotoxic secondary metabolites (Mukhopadhyay and Kumar, 2020; Ayyandurai *et al*., 2024). In addition, *Trichoderma* spp. is known to induce systemic resistance in host plants and promote root growth, enhancing tolerance to nematode-induced stress.

Historically, early applications in the 20th century reported reduced egg production of *Meloidogyne arenaria* following soil amendments with *T. harzianum* and *T. koningii* (Szabó *et al*., 2012). Similarly, integrated applications of *T. harzianum* and neem cake suppressed *Tylenchulus semipenetrans* populations in citrus (El-Deriny *et al*., 2022), while greenhouse trials demonstrated effective suppression of *Meloidogyne javanica* using *T. lignorum* and *T. harzianum* isolates (Mukhtar *et al*., 2021). In more recent research, several *Trichoderma* species including *T. viride*, *T. harzianum*, and *T. longibrachiatum* have demonstrated strong antagonistic activity not only against PPN but also against major soilborne pathogens such as Fusarium, Pythium, Rhizoctonia, and *Sclerotium* spp. (Zhang *et al*., 2015; Guzmán-Guzmán *et al*., 2023). Notably, *T. longibrachiatum* has shown capacity to inhibit egg hatching of *Meloidogyne incognita* and *Heterodera avenae* (Zhang *et al*., 2015; 2017a). In trials in Kenya, *T. asperellum* and *T. viride* successfully colonized pineapple roots and suppressed nematode reproduction under greenhouse conditions (Kiriga *et al*., 2018).

In legumes such as pea, the biocontrol potential of *Trichoderma* spp. extends beyond nematode suppression. Field and greenhouse studies have reported reduced root galling, enhanced nodulation, and increased pod yields following applications of *T. harzianum* and *T. viride* (Singh *et al*., 2018; El-Nagdi *et al*., 2019; Khan *et al*., 2023b). These findings highlight the broad-spectrum utility of *Trichoderma* spp. as a sustainable biocontrol option for integrated nematode management in potato and pea cropping systems.

However, the practical deployment of *Trichoderma* spp. under field conditions remains a major constraint. Limitations in spore viability, inconsistent colonization of rhizospheres, and the need for repeated inoculations have limited its scalability (Kumar *et al*., 2023). To address this, recent innovations have turned to biodegradable banana fibre paper as a novel delivery platform (Pirzada *et al*., 2023). Originally developed to administer chemical nematicides at ultra-low doses, this fibre-based matrix also shows promise for biological formulations. The paper functions by physically disrupting nematode host-finding cues and chemically releasing embedded agents directly into the root zone, allowing for controlled, localized suppression (Ochola *et al*., 2022; Kamau *et al*., 2024). Previous studies with abamectin-treated paper demonstrated nematode suppression at rates 500 to 600 times lower than conventional application, significantly improving potato yields under field conditions (Kamau *et al*., 2024). Yet, the potential for banana fibre paper to deliver *Trichoderma* spp. and achieve comparable suppression, particularly in dual-crop systems involving peas, remains unexplored.

While potatoes have been a focal crop in nematode biocontrol research, peas have received much less attention, despite their nutritional importance, nitrogen-fixing potential, and vulnerability to multiple nematode species (Ndinya *et al*., 2020). Moreover, few studies have directly compared the performance of biological and chemical agents delivered through the same biodegradable platform. Comparing *Trichoderma* paper to fluopyram and abamectin papers under field conditions across different crops and locations is essential for informing integrated and scalable management solutions. Moreover, information is lacking on the repeated use of treated banana fibre paper over multiple consecutive cropping seasons.

This chapter therefore investigates the effect of four banana paper treatment types: fluopyram-, abamectin-, *Trichoderma*-treated and untreated paper, on the abundance and diversity of plant parasitic nematodes under potato and pea cultivation. The focus is on comparing suppressive efficacy, examining genus-level nematode responses, and evaluating the yield response of using chemical versus biological inputs. By integrating legumes into nematode field trials and testing biological control through an innovative, farmer-accessible delivery system, this study contributes important insights into the future of sustainable nematode management in mixed smallholder farming systems.

6.2 Materials and Methods

6.2.1 Experimental design

Two crop trials using potatoes and peas were set up at two locations each: one in Kinangop, Nyandarua county (0.72786° S, 36.6542° E) and a second location in Borabu, Nyamira county (0.8082° S, 34.9668° E). The choice of location was based on contrasting nematode and agroecological conditions. Nyandarua County, identified as a hotspot for potato cyst nematode (PCN) infestations, provided a representative site for assessing treatment effects under high nematode pressure. In contrast, Nyamira County served as a comparative site with no known PCN incidence, allowing evaluation of treatment performance under low or absent cyst nematode conditions.

At each location, five treatments were compared: abamectin-treated paper (chemical based), fluopyram-treated paper (chemical based), *Trichoderma*-treated paper (biological based), untreated paper (as a positive control) and no paper (as an absolute control). Abamectin and fluopyram banana paper sheets came pretreated from North Carolina State University (NCSU, Raleigh, North Carolina, USA). For *Trichoderma* paper, untreated paper was used and along with *T. asperellum* suspension (1.5×10^4 cfu ml^{-1}). The commercial *T. asperellum* product (Sustain OD®, RealIPM Ltd., Thika, Kenya) was supplied at a concentration of 1.0×10^9 cfu ml^{-1} and diluted approximately 1:67 000 with water to obtain a working suspension of 1.5×10^4 cfu ml^{-1}. Each planting hole received 300 ml of this suspension prior to light soil covering (Kamau *et al*., 2024).

Potatoes (cv Shangi) were planted in plots of 3.0 × 3.75 m, with 50 tubers planted per plot at a spacing of 30 cm between plants and 75 cm between rows at a depth of 5 cm, resulting in a plant population density of 44,444 plants ha^{-1}. Each potato tuber was loosely wrapped with banana paper measuring 10 cm x 12.5 cm and placed in the planting hole (Fig. 6.1).

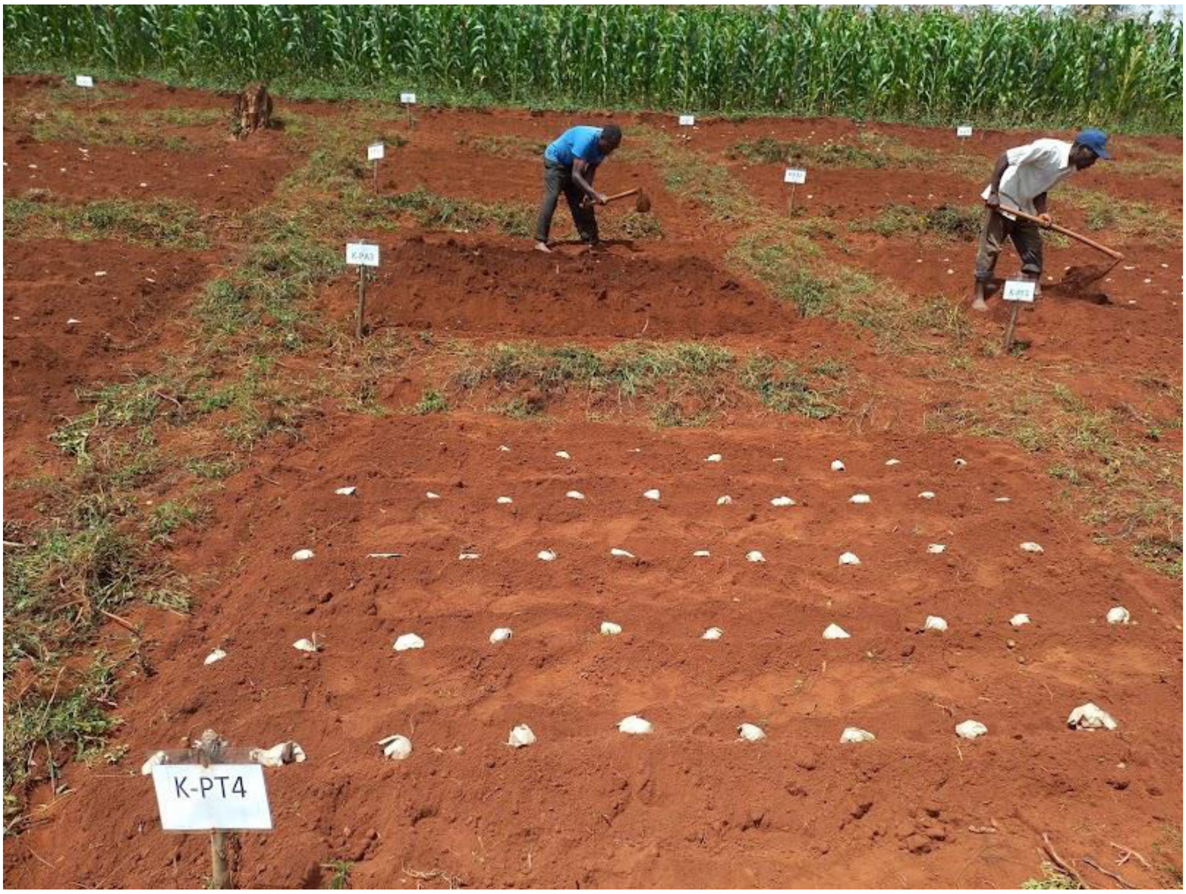

Figure 6.1 Wrapped potato tubers in Nyamira county at planting time

For green peas, Greenfeast pea seeds were sourced from Kenya seed company. Two seeds were wrapped in cut banana paper measuring 3 cm × 3 cm and planted at a spacing of 10 cm between plants and 75 cm between rows (Fig. 6.2). A total of three hundred (300) seeds were planted on each 3 × 4 m plots, giving a plant density of 133,333 plants ha^{-1}. A buffer zone of 0.75 m separated the experimental units. Trellises were put in place after 14 days to support the plants.

Figure 6.2 Wrapped pea grains in Nyandarua county during planting

For both crops and at both sites, the treatment was replicated four times and arranged in a randomized complete block design (RCBD). Routine agronomic practices were conducted regularly. Diammonium phosphate (DAP) was applied at a rate of 50 kg/ha during planting. After 30 days, calcium ammonium nitrate (CAN) fertilizer at a rate of 50 kg/ha was applied as a top dress to boost crop production. Pests and diseases were managed using chemical pesticides. The fungicides Ridomil Gold® (active ingredients: Metalaxyl-M 40 g Kg^{-1} and Mancozeb 640 g Kg^{-1}; Syngenta, Kenya) and Ortiva 250 SC® (active ingredient: 250 g L^{-1} Azoxystrobin; Syngenta, Kenya), were applied bi-weekly and interchangeably starting from day 45 until dehaulming of potatoes and harvest of peas. The trials were rainfed, with hand-weeding undertaken at 30-day intervals (twice each season).

Potato trials were repeated over four and three consecutive cropping seasons in Nyandarua and Nyamira, respectively, and details of the rainfall and temperature conditions are as depicted in Table 6.1. Initially, the study was established in Kakamega County but was abandoned due to ownership and leasing issues after the first cropping season, relocating it to Nyamira County, where three cropping seasons were completed. In contrast, trials in Nyandarua proceeded for four seasons as originally planned. Pea trials were conducted for three seasons at each location. For both crops, trials were terminated 90 days after planting. Upon harvest, plots were immediately prepared manually for the next cropping season, ensuring minimal delay before replanting with the same crop and treatment type.

Table 6.1 Seasonal rainfall and mean temperature recorded during the trial in Nyandarua and Nyamira

Site	Season	Rain	Period	Rainfall (mm)	Mean temperature
Nyandarua	Season 1	Short rains	Nov '22 - Jan '23	590.73	16.27˚C
	Season 2	Long rains	Mar - May '23	533.83	17.30˚C
	Season 3	Intermediate	Jul - Sep '23	134.7	16.64˚C
	Season 4	Short rains	Nov '23 - Jan '24	510.75	16.73˚C
Nyamira	Season 1	Long rains	Mar - May '23	859.76	18.37˚C
	Season 2	Intermediate	Jul - Sep '23	636.93	17.30˚C
	Season 3	Short rains	Nov '23 - Jan '24	372.06	17.17˚C

6.2.2 Data collection

The procedures for collecting and processing plant-parasitic nematode data followed the methods outlined in Section 4.2.2. In brief, composite samples of at least 500 g soil and 50 g roots were collected from each plot and immediately transported to the laboratory in a cool box for processing. Extraction for vermiform nematodes followed the modified Baermann technique using 100 ml soil and 5 g root sub-samples (Coyne *et al*., 2018b). Nematode suspensions were reduced to 10 ml and nematodes counted from three × 2 ml aliquots using a stereo-microscope (Leica MZ12) and expressed per 100 ml soil or 5 g roots. For each sample, at least 100 nematodes were identified to genus level using a compound microscope (Carl-Zeiss). Nematode identification was based on morphological features (KSU, 2024). For potato cyst nematode extraction, the Fenwick can method was used with 200 ml of air-dried soil (EPPO, 2017). However, due to the low or absent recovery of cysts, the analysis proceeded to focus solely on vermiform nematodes.

Vegetative growth parameters were recorded approximately 45 days after planting, right after the vegetative growth stage in both potato and pea plots. For potatoes, five plants were randomly selected from each plot for measurement of plant height, canopy diameter, number of leaves and branches, leaf length and stem diameter. Plant height and canopy diameter were measured using a measuring tape (accurate to ±1 cm), while the number of leaves and branches were determined through manual counting. The length of the longest leaf on each plant was measured using a standard 30 cm ruler. Stem diameter was assessed 5 cm above the root collar using a string wrapped around the stem and then measured against a ruler. In pea plots, ten plants were randomly selected per plot and evaluated using the same procedure. Plant height, number of branches, number of leaves, and leaf length were measured as described for potato, and stem diameter was similarly recorded using a string and ruler.

At crop maturity, which occurred approximately 90 days after planting, yield assessments were carried out. For potatoes, total fresh tuber weight per plot was measured using a field-calibrated portable digital scale (20 kg capacity, ±0.1 kg accuracy). Yields were converted to tons per hectare (t ha^{-1}). Tuber grading was done manually based on market standards: marketable tubers were of adequate size and free from visible defects, while unmarketable tubers included undersized ones (commonly referred to as 'chatts'), deformed, rotting, or pest-damaged specimens.

Peas were harvested at physiological maturity by manually plucking mature green pods from plants. In each plot, the total number of pods was measured using a digital weighing scale and standardized to t ha^{-1}. Visual grading was conducted immediately after harvest, with marketable pods defined as well-filled, fresh, and undamaged. Unmarketable pods were those with poor grain fill or physical damage from biotic or abiotic factors.

6.2.3 Data analysis

Data for abundance and diversity of plant parasitic nematodes, crop growth parameters and yield were first tested for normality using Shapiro-Wilk test. Where necessary, they were transformed to natural log. Data were then subjected to generalized linear mixed-effects model analysis of variance (ANOVA). Banana paper treatments were considered as fixed factor while study location was considered as a random factor in the "lme4" package (Bates *et al*., 2015) using the model: $T_{ij} = \mu + O_i + M_j + (OM)_{ij} + \varepsilon_i$ Where: T_{ij} is the total observation, μ is the mean, O_i is the ith banana paper treatment effect, M_j is jth location treatment effect, $(OM)_{ij}$ is the interaction between banana paper and location, and ε_{ij} is the variation due to random error. Since there were significant differences between the host crops, data for potatoes and peas were presented separately. Data for each location was also reported separately owing to the different timelines during which the trials were conducted. Means were separated using Tukey's test at $p < 0.05$ confidence level. All analyses were performed using R version 4.2.3 statistical package (R Core Team, 2023).

6.3 Results

6.3.1 Plant parasitic nematodes associated with potato and pea agroecosystems

A total of 19 taxa of plant parasitic nematodes, belonging to eleven families, were detected during the entire study period. All 19 genera were recorded from potatoes (18 in Nyandarua and 9 in Nyamira). The mean density of the nematode genera varied across study sites for the initial (Pi, $\chi^2 \geq 1926.8$, $p < 0.001$) and final (Pf, $\chi^2 \geq 3721.6$, $p < 0.001$) nematode counts, respectively. For potato trials, the most commonly detected economically important nematode species were *Globodera* spp. (61 nematodes 100 ml^{-1} soil) in Nyandarua, and *Trichodorus* spp. (223 nematodes 100 ml^{-1} soil) in Nyamira (Table 6.2).

Table 6.2. Prevalent plant parasitic nematodes affecting potatoes (cv. Shangi) in Nyandarua and Nyamira counties of Kenya

Family	Genus	[1]Nyandarua (Pi)	[2]Nyandarua (Pf)	Nyamira (Pi)	Nyamira (Pf)
Aphelenchoidae	*Aphelenchoides* spp.	31.23 ± 4.32 c	6.90 ± 1.88 def	63.37 ± 5.76 c	63.20 ± 9.15 b
Aphelenchoididae	*Aphelenchus* spp.	48.54 ± 4.52 b	19.50 ± 5.06 cdef	2.57 ± 1.35 e	3.45 ± 2.79 c
Belonolaimidae	*Tylenchorhynchus* spp.	9.45 ± 2.09 de	2.15 ± 1.11 f	0 ± 0 e	0 ± 0 c
Criconematidae	*Criconema* spp.	2.59 ± 0.98 e	1.50 ± 1.18 f	0 ± 0 e	0 ± 0 c
Hemicycliophoridae	*Hemicycliophora* spp.	35.99 ± 4.36 c	16.20 ± 6.73 cdef	0 ± 0 e	0 ± 0 c
Heteroderidae	*Globodera* spp.	86.71 ± 7.86 a	61.4 ± 6.88 a	0 ± 0 e	0 ± 0 c
	Meloidogyne spp.	13.88 ± 1.74 d	3.55 ± 1.39 f	163.30 ± 19.81 b	11.20 ± 11.20 c
Hoplolaimidae	*Helicotylenchus* spp.	9.28 ± 2.13 de	0 ± 0 f	0 ± 0 e	0 ± 0 c
	Hoplolaimus spp.	1.34 ± 0.71 e	5.35 ± 2.70 ef	0 ± 0 e	0 ± 0 c
	Rotylenchulus spp.	0.33 ± 0.19 e	1.30 ± 0.72 f	0 ± 0 e	0 ± 0 c
	Rotylenchus spp.	15.25 ± 2.18 d	27.50 ± 6.89 cd	0 ± 0 e	0 ± 0 c
	Scutellonema spp.	0.85 ± 0.45 e	3.40 ± 1.72 f	0 ± 0 e	0 ± 0 c
	Trichotylenchus spp.	0 ± 0 e	0 ± 0 f	10.17 ± 2.22 de	30.50 ± 3.66 bc
Longidoridae	*Longidorus* spp.	10.15 ± 2.45 de	3.65 ± 1.55 f	0 ± 0 e	0 ± 0 c
	Xiphinema spp.	1.90 ± 0.76 e	7.60 ± 2.7 def	11.27 ± 2.05 de	13.05 ± 3.82 c
Pratylenchidae	*Pratylenchus* spp.	6.45 ± 2.63 de	25.80 ± 9.43 cde	73.87 ± 16.82 c	221.60 ± 30.23 a
Trichodoridae	*Trichodorus* spp.	0 ± 0 e	0 ± 0 f	223.17 ± 10.57 a	231.75 ± 19.94 a
Tylenchidae	*Filenchus* spp.	42.06 ± 3.22 bc	60.10 ± 10.11 ab	246.43 ± 10.37 a	246.70 ± 18.08 a
	Tylenchus spp.	33.60 ± 4.77 c	38.15 ± 13.87 bc	31.53 ± 1.99 d	32.50 ± 4.08 bc
		***	***	***	***

[1]Means are recorded at commencement of trial period in the first cropping season (Pi)
[2]Means are recorded at end of trial period in the last cropping season (Pf)
Means followed by the same lowercase letters in each column are not significant at $p \leq 0.05$

For peas, only 13 genera were identified (11 in Nyandarua and 8 in Nyamira). *Meloidogyne* spp. was the predominantly important nematode genera in both Nyandarua (42 nematodes 100 ml^{-1} soil) and Nyamira (110 nematodes 100 ml^{-1} soil), respectively (Table 6.3).

Table 6.3. Prevalent plant parasitic nematodes affecting peas (cv. Greenfeast) in Nyandarua and Nyamira counties of Kenya

Family	Genus	[1]Nyandarua (Pi)	[2]Nyandarua (Pf)	Nyamira (Pi)	Nyamira (Pf)
Aphelenchoidae	*Aphelenchoides* spp.	54.00 ± 4.82 b	53.65 ± 6.44 ab	72.67 ± 3.58 b	86.55 ± 7.09 b
Aphelenchoididae	*Aphelenchus* spp.	37.53 ± 4.73 c	39.95 ± 7.29 b	0 ± 0 e	0 ± 0 d
Criconematidae	*Criconema* spp.	11.37 ± 2.52 d	15.60 ± 4.77 c	6.88 ± 2.08 e	13.30 ± 4.91 d
Heteroderidae	*Globodera* spp.	6.98 ± 2.01 d	9.55 ± 3.99 c	0 ± 0 e	0 ± 0 d
	Meloidogyne spp.	41.98 ± 4.06 bc	50.45 ± 4.58 b	110.33 ± 4.98 a	133.40 ± 8.16 a
Hoplolaimidae	*Helicotylenchus* spp.	35.22 ± 3.21 c	49.35 ± 5.95 b	39.65 ± 4.44 b	0 ± 0 d
	Rotylenchulus spp.	10.22 ± 1.75 d	16.45 ± 3.52 c	0 ± 0 c	0 ± 0 d
	Rotylenchus spp.	12.35 ± 2.36 d	17.50 ± 4.95 c	10.25 ± 3.06 c	0 ± 0 d
	Scutellonema spp.	0 ± 0 d	0 ± 0 c	15.2 ± 4.24 c	0 ± 0 d
	Trichotylenchus spp.	0 ± 0 d	0 ± 0 c	0 ± 0 e	0 ± 0 d
Longidoridae	*Longidorus* spp.	0 ± 0 d	0 ± 0 c	0.93 ± 0.93 e	2.80 ± 2.80 d
Pratylenchidae	*Pratylenchus* spp.	1.88 ± 0.73 d	1.70 ± 1.31 c	76.35 ± 4.94 b	87.65 ± 9.58 b
Tylenchidae	*Filenchus* spp.	86.73 ± 5.12 a	74.70 ± 4.04 a	52.63 ± 3.46 c	61.55 ± 6.30 c
	Tylenchus spp.	95.55 ± 6 a	58.30 ± 3.90 ab	36.85 ± 3.76 d	46.55 ± 8.05 c

[1]Means are recorded at commencement of trial period in the first cropping season (Pi)
[2]Means are recorded at end of trial period in the last cropping season (Pf);
Means followed by the same lowercase letters in each column are not significant at $p \leq 0.05$

6.3.2 Relative abundance of plant parasitic nematodes among banana paper treatments

At each trial location, there was no interaction of season and treatment ($\chi^2 \geq 1.9$, $p = 0.38$) on nematode density for either soil or roots from potato, therefore, data were pooled across seasons prior to further analysis. In Nyandarua, the nematode density in both potato roots and soil varied significantly ($\chi^2 \geq 12.21$, $p = 0.01$) across treatments (Table 6.4). Abamectin paper recorded the lowest nematode density (258 nematodes 100 ml^{-1} soil) in soil, while lower densities (12 nematodes 5 g^{-1} roots) in roots were found in fluopyram paper plots. In Nyamira, both fluopyram- and *Trichoderma*-treated paper recorded lower ($\chi^2 \geq 71.47$, $p < 0.001$) nematode densities in both soil and roots, relative to the control.

At both trial sites, the nematode density in peas did not vary by season, nor was there a significant season-treatment interaction ($p \geq 0.95$). Nematode densities varied among the treatments in soil and roots ($\chi^2 \geq 75.43$, $p < 0.001$ in Nyandarua; $\chi^2 \geq 28.96$, $p < 0.001$ in Nyamira) (Table 6.4). Abamectin paper and untreated paper recorded significantly lower nematode densities in soil samples from Nyandarua (< 433 nematodes 100 ml^{-1} soil) than control (> 608 nematodes 100 ml^{-1} soil). The root samples had none of the treatments outperform the control with a similar outcome in Nyamira soil samples. However, all treatments had lower nematode densities than the control plots in root samples from Nyamira.

Table 6.4. Effect of banana fibre paper on the mean abundance of plant parasitic nematodes infecting potatoes (cv. Shangi) and peas (cv. Greenfeast) in field trials at Nyandarua and Nyamira counties, Kenya

Trial	Site	Treatment	Soil[1]	Roots[2]
Potato	Nyandarua[3]	Abamectin	257.88 ± 40.95 b	18.63 ± 2.33 ab
		Control	319.75 ± 44.85 a	22.19 ± 3.33 a
		Fluopyram	289.38 ± 31.99 ab	11.75 ± 2.15 b
		Trichoderma	298.75 ± 36.59 ab	19.50 ± 2.43 ab
		Untreated	316.67 ± 26.94 a	14.44 ± 1.45 ab
	Nyamira[4]	Abamectin	882.58 ± 24.84 ab	386.25 ± 14.32 a
		Control	951.75 ± 37.68 a	402.83 ± 9.72 a
		Fluopyram	621.58 ± 28.97 c	56.00 ± 3.93 c
		Trichoderma	770.75 ± 15.06 b	233.92 ± 8.10 b
		Untreated	910.08 ± 42.53 a	52.75 ± 3.52 c
Pea	Nyandarua	Abamectin	432.31 ± 86.01 b	34.58 ± 5.86 b
		Control	608.56 ± 111.37 a	28.08 ± 5.65 bc
		Fluopyram	648.58 ± 130.89 a	26.25 ± 5.03 bc
		Trichoderma	605.17 ± 110.68 a	49.25 ± 9.54 a
		Untreated	334.25 ± 40.57 b	20.67 ± 3.08 c
	Nyamira	Abamectin	449.83 ± 58.73 b	118.33 ± 16.81 b
		Control	549.17 ± 64.07 ab	166.50 ± 42.92 a
		Fluopyram	667.00 ± 133.21 a	115.00 ± 14.30 b
		Trichoderma	545.08 ± 128.84 ab	141.67 ± 9.52 b
		Untreated	437.92 ± 68.42 b	117.42 ± 22.87 b

[1] Soil: samples obtained from composite sample of 100 ml
[2] Root: samples obtained from composite sample of 5 g
[3] Means and standard error of mean are pooled from four consecutive cropping seasons
[4] Means and standard error of mean are pooled from three consecutive cropping seasons
Means followed by the same lowercase letters in each column are not significant at $p \leq 0.05$

6.3.3 Effect of banana fibre paper treatments on crop growth

In the Nyandarua trial, potato growth traits differed among treatments ($\chi^2 \geq 75.43$, $p < 0.001$). Plant height, leaf number, leaf length and stem diameter were all influenced by treatment, with overall greater plant growth observed under abamectin-treated paper than the control (Table 6.5). However, the number of branches and canopy diameter remained similar across treatments. Meanwhile in Nyamira, the third cropping season was affected by severe drought resulting in crop failure. Consequently, data from this season was not included in the analysis. However, from the first two seasons, *Trichoderma*- and fluopyram-treated paper resulted in higher ($\chi^2 \geq 7.52$, $p < 0.05$) values of plant height, leaf count, leaf length, and canopy diameter but a similar number of branches and stem diameter.

Table 6.5. Effect of banana fibre paper on crop growth parameters of potatoes (cv. Shangi) in Nyandarua and Nyamira counties, Kenya

Site	Treatment	Plant height	No. of branches	No. of leaves	Length of leaves	Stem diameter	Canopy diameter
Nyandarua[1]	Abamectin	45.08 ± 2.60 a	3.23 ± 0.22 a	140.98 ± 15.03 ab	6.88 ± 0.25 ab	2.59 ± 0.10 a	46.15 ± 2.41 a
	Control	36.00 ± 2.62 b	3.27 ± 0.28 a	168.43 ± 13.5 a	6.34 ± 0.28 ab	1.75 ± 0.10 c	44.93 ± 2.58 a
	Fluopyram	41.42 ± 3.10 ab	3.37 ± 0.28 a	104.23 ± 9.37 c	6.33 ± 0.30 ab	2.10 ± 0.09 abc	39.85 ± 2.37 a
	Trichoderma	41.38 ± 3.07 ab	3.35 ± 0.24 a	122.62 ± 9.15 bc	7.04 ± 0.15 a	2.40± 0.10 abc	42.90 ± 2.50 a
	Untreated	32.99 ± 2.71 b	2.88 ± 0.27 a	119.23 ± 11.99 bc	6.11 ± 0.26 b	1.92 ± 0.10 bc	38.31 ± 2.49 a
Nyamira[2]	Abamectin	28.78 ± 2.36 ab	3.18 ± 0.26 a	48.68 ± 3.77 c	5.94 ± 0.06 b	1.97 ± 0.11 a	55.23 ± 2.21 ab
	Control	27.78 ± 2.69 b	2.98 ± 0.28 a	90.30 ± 3.86 b	6.15 ± 0.10 ab	2.20 ± 0.10 a	53.18 ± 2.47 b
	Fluopyram	29.28 ± 2.89 ab	3.15 ± 0.28 a	119.00 ± 5.04 a	6.40 ± 0.23 ab	1.86 ± 0.14 a	49.18 ± 3.21 b
	Trichoderma	38.73 ± 2.97 a	3.63 ± 0.31 a	98.10 ± 3.52 b	6.60 ± 0.11 a	2.31 ± 0.14 a	63.78 ± 1.33 a
	Untreated	31.48 ± 2.79 ab	2.60 ± 0.23 a	53.70 ± 4.48 c	6.00 ± 0.05 b	1.90 ± 0.12 a	46.83 ± 3.86 b

Data represents average mean of five plants per replicate with four replicates per treatment
[1]Means are pooled from three consecutive cropping seasons
[2]Means are pooled from two consecutive cropping seasons
Means followed by the same lowercase letters in each column grouping are not significant at $p \leq 0.05$

In pea trials at Nyandarua, only plant height differed ($\chi^2 \geq 22.85$, $p < 0.001$) among the treatments, with fluopyram-treated paper recording taller plants (52 cm) than in the control (43 cm) (Table 6.6). At the Nyamira site, all growth parameters varied widely ($\chi^2 \geq 21.06$, $p < 0.001$), where fluopyram- and *Trichoderma*-treated paper consistently resulted in higher plant growth than in control plots.

Table 6.6 Effect of banana fibre paper on crop growth parameters of peas (cv. Greenfeast) in Nyandarua and Nyamira counties, Kenya

Site	Treatment	Plant height	No. of branches	No. of leaves	Length of leaves	Stem diameter
Nyandarua[1]	Abamectin	45.05 ± 1.41 b	8.03 ± 0.18 a	45.68 ± 0.86 a	4.92 ± 0.22 a	2.81 ± 0.09 a
	Control	43.16 ± 1.87 b	7.85 ± 0.18 a	45.83 ± 1.06 a	4.43 ± 0.20 a	2.72 ± 0.10 a
	Fluopyram	51.88 ± 2.02 a	8.21 ± 0.22 a	46.87 ± 0.94 a	5.06 ± 0.22 a	2.68 ± 0.09 a
	Trichoderma	43.96 ± 1.89 b	8.12 ± 0.26 a	43.63 ± 1.23 a	4.93 ± 0.20 a	2.99 ± 0.10 a
	Untreated	47.58 ± 1.66 ab	7.88 ± 0.22 a	44.00 ± 1.19 a	4.29 ± 0.21 a	2.76 ± 0.09 a
Nyamira[2]	Abamectin	53.75 ± 1.74 c	8.51 ± 0.21 b	49.90 ± 0.97 ab	5.20 ± 0.23 a	2.89 ± 0.11 a
	Control	63.00 ± 2.07 ab	8.06 ± 0.21 b	49.34 ± 0.99 ab	3.41 ± 0.23 b	2.13 ± 0.08 b
	Fluopyram	67.71 ± 1.53 a	8.65 ± 0.24 b	47.44 ± 1.30 b	5.11 ± 0.27 a	2.68 ± 0.11 a
	Trichoderma	63.06 ± 2.49 ab	9.58 ± 0.25 a	52.39 ± 0.99 a	5.78 ± 0.22 a	3.00 ± 0.09 a
	Untreated	58.53 ± 1.88 bc	8.16 ± 0.24 b	45.94 ± 1.08 b	3.63 ± 0.24 b	2.66 ± 0.10 a

Data represents average mean of 10 plants per replicate with four replicates per treatment
[1]Means are pooled from three consecutive cropping seasons
[2]Means are pooled from two consecutive cropping seasons
Means followed by the same letter(s) along the columns for each county are not statistically different at $p < 0.05$

6.3.4 Effect of banana fibre paper treatments on yield

Potato yields differed among the treatments ($\chi^2 \geq 12.28$, $p < 0.02$) during each season ($\chi^2 \geq 75.99$, $p < 0.001$) with significant treatment by season interactions ($\chi^2 \geq 45.08$, $p < 0.001$) at both sites. Results for each season were therefore analyzed and presented separately. In Nyandarua, fluopyram- and *Trichoderma*-treated paper resulted in greater marketable yields than the control in the first and second cropping seasons. However, no treatment performed better than the control in the third and fourth seasons (Fig. 6.3).

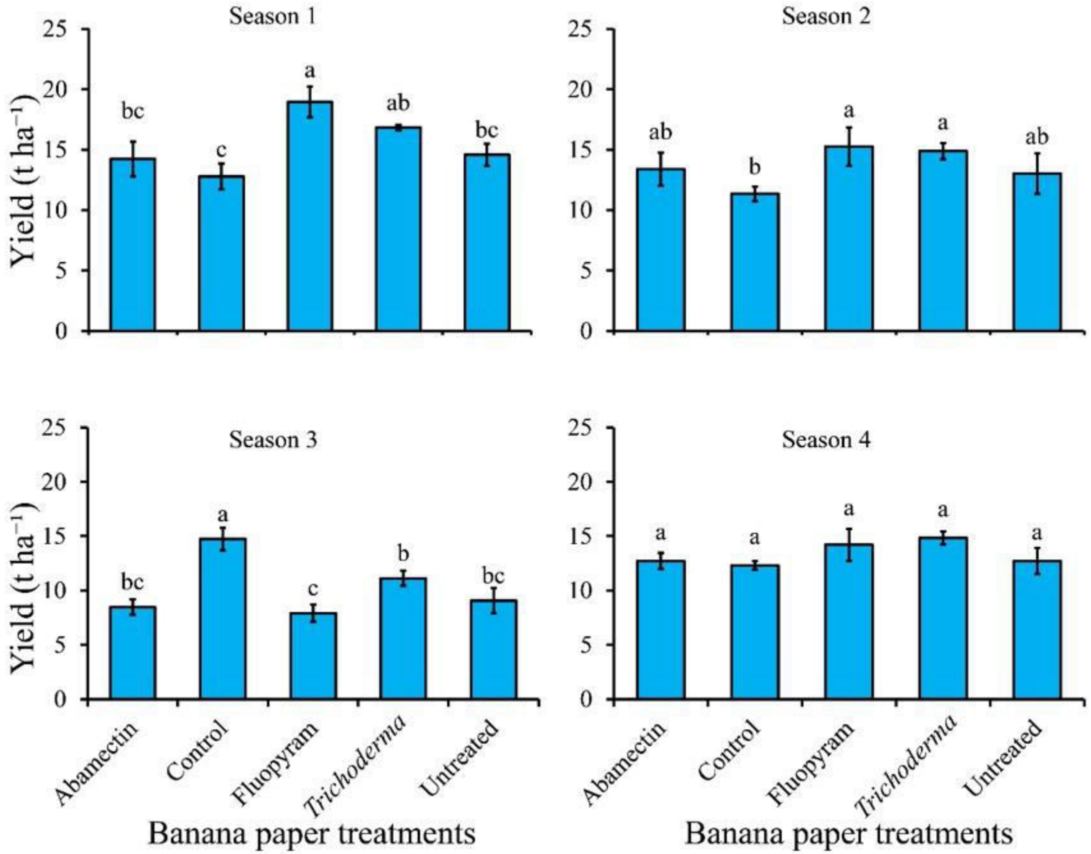

Figure 6.3 Effect of banana paper treatments on the yield of potatoes (cv. Shangi) in Nyandarua county, Kenya.

Season 1 = May to July (long rains), Season 2 = September to November (short rains); Season 3 = January to April (intermediate rains); Season 4 = May to July (long rains). Bars followed by the same letter are not significantly different at $p < 0.05$.

In Nyamira county, all treatments led to higher yields than the control in the first two seasons, where plots with *Trichoderma*-treated (9.22 and 8.91 t ha^{-1} in season one and two, respectively) and fluopyram-treated paper (8.7 and 8.03 t ha^{-1} in season one and two, respectively) had double the potato tuber weight, compared to the control (4.47 and 4.37 t ha^{-1} in season one and two, respectively) (Fig. 6.4).

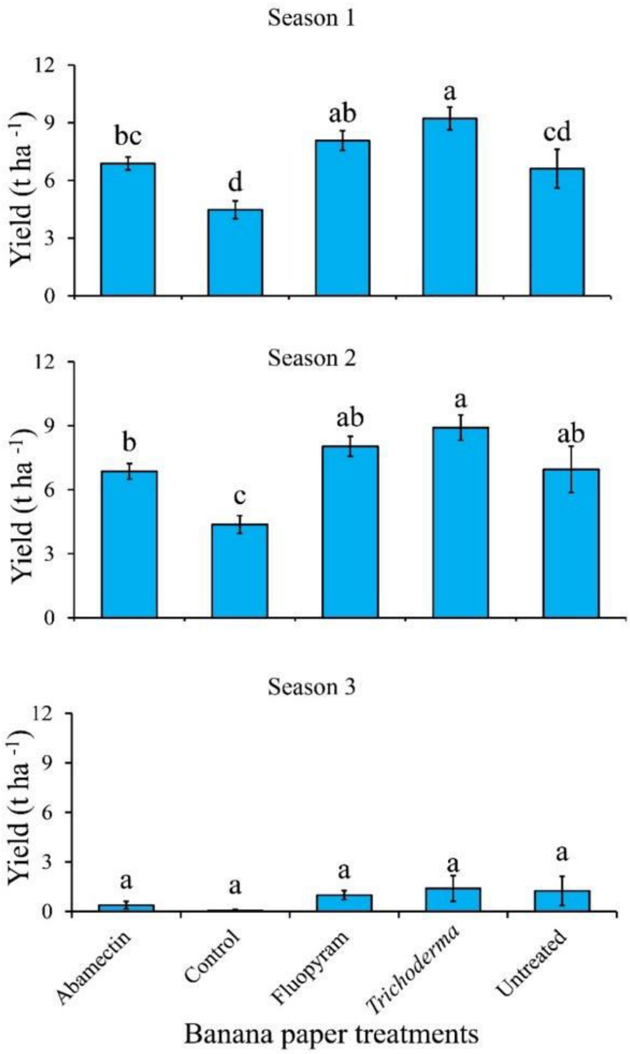

Figure 6.4 Effect of banana paper treatments on the yield of potatoes (cv. Shangi) in Nyamira county, Kenya.

Season 1 = September to November (short rains); Season 2 = January to April (intermediate rains); Season 3 = May to July (long rains). Bars followed by the same letter are not significantly different at $p < 0.05$.

In addition to total yield, the quality of potato tubers, categorized as marketable and unmarketable, differed significantly among treatments ($\chi^2 \geq 12.28$, $p < 0.05$). There were no significant seasonal variations ($\chi^2 \geq 1.7$, $p = 0.41$) at either site and therefore data were pooled cropping seasons (Appendix viii). Across both Nyandarua and Nyamira sites, all treated plots produced a higher number of marketable tubers compared to the control. In Nyandarua, *Trichoderma*-treated and fluopyram-treated plots resulted in the greatest yield of marketable tubers and the lowest levels of unmarketable ones, indicating improved tuber quality relative to the control (Fig. 6.5). Similarly, in Nyamira, abamectin-, fluopyram-, and *Trichoderma*-treated plots all outperformed the control in marketable tubers.

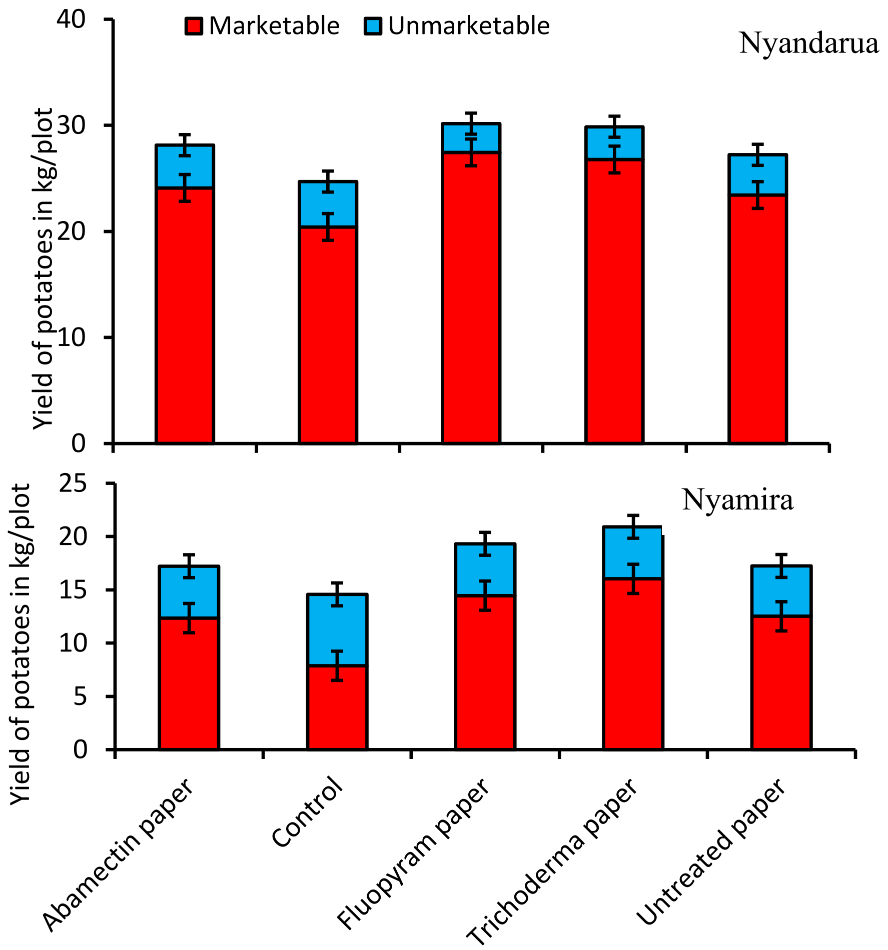

Figure 6.5 Effect of banana paper treatments on the quality of potatoes (cv. Shangi) in Nyandarua and Nyamira counties, Kenya.

Means represent pooled data for three cropping seasons in Nyandarua, and two seasons in Nyamira.

For pea trials, no yield differences were observed in Nyandarua or Nyamira across seasons ($\chi^2 \leq 7.42$, $p > 0.12$) and therefore data was pooled for seasons. Among the treatments, no variations occurred at either site for total yield, marketable yield or unmarketable yield ($p > 0.05$) (Table 6.7)

Table 6.7 Yield quantity and quality across banana paper treatments in Nyandarua and Nyamira, Kenya

Yield	Treatment	Nyandarua ($t\ ha^{-1}$)	Nyamira
Total yield	Abamectin-paper	2.13 ± 0.50	1.72 ± 0.46
	Control	2.18 ± 0.50	1.77 ± 0.43
	Fluopyram-paper	2.33 ± 0.6	1.79 ± 0.47
	Trichoderma-paper	2.49 ± 0.75	2.10 ± 0.65
	Untreated paper	1.97 ± 0.46	1.42 ± 0.41
Marketable yield	Abamectin-paper	1.51 ± 0.39	1.29 ± 0.37
	Control	1.53 ± 0.39	1.13 ± 0.38
	Fluopyram-paper	1.68 ± 0.46	1.23 ± 0.40
	Trichoderma-paper	1.78 ± 0.57	1.46 ± 0.55
	Untreated paper	1.31 ± 0.36	0.94 ± 0.34
Unmarketable yield	Abamectin-paper	0.61 ± 0.12	0.43 ± 0.10
	Control	0.65 ± 0.12	0.64 ± 0.08
	Fluopyram-paper	0.65 ± 0.14	0.56 ± 0.09
	Trichoderma-paper	0.71 ± 0.19	0.64 ± 0.11
	Untreated paper	0.66 ± 0.12	0.48 ± 0.08

Means are pooled from three consecutive cropping seasons

6.4 Discussion

This study builds upon previous studies that have shown banana fibre paper to be a viable carrier medium for nematicidal agents, notably abamectin, in reducing plant parasitic nematode populations in root and tuber crops such as potato and yam (Ochola *et al*., 2022; Dedehouanou *et al*., 2022; Pirzada *et al*., 2023; Kamau *et al*., 2024). Those studies demonstrated that impregnating abamectin in processed banana waste-based paper resulted in superior nematode control compared to conventional direct application of the same chemical. Building on this, the research evaluated banana paper as a carrier not only for abamectin and fluopyram as nematicides, but also *Trichoderma* spp., a biological control agent, in potato and pea systems across multiple seasons. To isolate the performance of the paper-based system, standard (non-paper) applications of fluopyram, abamectin, and *Trichoderma* were not included. These conventional approaches have been extensively validated in other studies. The novelty in this approach lay in determining whether micro-dosed, paper-delivered formulations could match or surpass standard treatments in nematode suppression while minimizing environmental contamination and input costs.

Among the major nematode pests observed, *Globodera* spp. and *Trichodorus* spp. dominated the Nyandarua and Nyamira sites respectively. *Globodera rostochiensis* and *G. pallida* are among the most destructive potato pests globally, with the potential to reduce yields by up to 80% (Coyne *et al*., 2018a; Mburu *et al*., 2020; Abrantes *et al*., 2023; Onditi and Whitworth, 2024). Meanwhile, *Trichodorus* spp., known vectors of tobacco rattle virus (TRV), are linked to corky ringspot disease in potato—a condition that can severely compromise market value (Bairwa *et al*., 2022). Although initial and final nematode population densities (Pi) were relatively similar in aggregate, paper-treated plots generally showed lower densities than controls. This suggests a broad-spectrum but moderate suppressive effect by banana paper treatments across multiple nematode genera, without species-specific targeting. Notably, *Globodera* spp. were only found in Nyandarua, consistent with their limited geographical spread in Kenya's major potato zones (Mburu *et al*., 2020).

Meloidogyne spp., while globally significant in potato production (Lima *et al*., 2018; Pulavarty *et al*., 2021), were observed at low levels in this study. This could be a result of using land that had remained fallow for over five years prior to trial initiation. In contrast, *Meloidogyne* and *Pratylenchus* spp. were more prevalent in the pea trials. These species are well-documented pathogens of pea crops in tropical and temperate zones (Riga *et al*., 2008; Upadhaya *et al*., 2019; Youssef and El-Nagdi, 2019). *Pratylenchus* spp., particularly *P. thornei* and *P. neglectus*, are frequently associated with legumes and have been reported as key nematode pests in pea-based systems (Kandel *et al*., 2018).

Nematode suppression was most consistent in plots treated with fluopyram-, *Trichoderma*-, and abamectin-impregnated banana paper. Fluopyram's nematicidal activity has been attributed to its inhibition of succinate dehydrogenase, an enzyme central to ATP synthesis and nematode energy metabolism (Faske and Hurd, 2015; Jones *et al*., 2017; Ji *et al*., 2019; Hawk and Faske, 2020). Even at low concentrations, fluopyram has been shown to immobilize nematodes effectively (Beeman and Tylka, 2018; Schleker *et al*., 2022). *Trichoderma* spp. operate through multiple mechanisms including parasitism, antibiosis, enzymatic degradation of nematode eggs and juveniles, and stimulation of plant defences (Zhang *et al*., 2015; Singh *et al*., 2018; Poveda *et al*., 2020; Asghar *et al*., 2024). Some strains, such as *T. asperellum*, are highly effective against *Meloidogyne javanica* due to their potent egg-parasitic capacity (Hemeda and El-Deeb, 2019; dos Santos Pereira *et al*., 2021).

Abamectin also showed consistent nematode suppression in the trials, echoing earlier studies that demonstrated its potency even at very low concentrations (Faske and Starr, 2006; Cao *et al*., 2015; 2016; d'Errico *et al*., 2017; Ochola *et al*., 2022; Kamau *et al*., 2024). Its neurotoxic mechanism leads to irreversible paralysis in nematodes, preventing feeding and reproduction. Interestingly, fluopyram outperformed abamectin in some instances, while the reverse held true in others, highlighting complementary modes of action that could be synergistically harnessed through combination treatments on banana fibre paper.

Even without the addition of nematicides, untreated banana-fibre paper contributed to both nematode suppression and modest yield enhancement. Across the field trials, plots with untreated paper showed intermediate reductions in PPN densities relative to fully untreated controls, possibly by restricting juvenile mobility or modifying root exudate interactions, consistent with earlier studies on banana-paper wrap technologies (Ochola *et al*., 2022). These observations align with reports that biodegradable ligno-cellulosic wraps can limit nematode penetration and provide incremental yield benefits in smallholder systems (Pirzada *et al*., 2023; Kamau *et al*., 2024). Although its impact was less pronounced than that of nematicide-treated papers, untreated paper remains a promising low-input approach or complementary tool in integrated nematode management strategies.

In terms of yield outcomes, fluopyram- and *Trichoderma*-treated banana paper consistently improved potato growth and tuber yield compared to the control during the first two cropping seasons in Nyandarua. These benefits were not sustained into seasons three and four. This decline could stem from environmental variability, continuous cropping, or microbial competition affecting treatment performance (Larkin *et al*., 2017; Qin *et al*., 2022; Petrushin *et al*., 2024). Additionally, residual impacts of fluopyram on beneficial soil microbes may have compromised longer-term plant health and nutrient cycling. This underscores the importance of evaluating ecological trade-offs in nematode control strategies.

In contrast to findings by Mahmoud *et al*. (2023), who reported yield improvements in peas using bio-fertilizers, no yield gains were observed in the pea trials. This may reflect the limited effectiveness of seed wrapping using banana paper for small-seeded crops. Traditional enhancement methods such as seed soaking or coating (Rocha *et al*., 2019; Lamichhane *et al*., 2022; Mendoza-Figueroa *et al*., 2024) may offer more uniform delivery. Wrapping individual seeds was both time-intensive and potentially inconsistent in dosage. To overcome this, current research is exploring banana fibre-derived pellets as an alternative means of delivering nematicides and microbes to grain crops more effectively and efficiently.

CHAPTER SEVEN: SOIL HEALTH RESPONSES TO BANANA FIBRE PAPER: INSIGHTS FROM FREE-LIVING NEMATODES AND MICROBIAL INDICATORS

7.1 Introduction

Soil health is a foundational element of agricultural productivity and sustainability, particularly in smallholder systems where chemical input levels are often constrained and natural biological processes underpin crop resilience (Van Bruggen and Finckh, 2016). In recent years, attention has increasingly shifted toward assessing not only the yield outcomes of pest management interventions but also their broader ecological footprint, especially their impact on soil biota that drive nutrient cycling, suppress pathogens, and maintain soil structure (Neher, 2010; Larkin *et al*., 2017). This paradigm shift is particularly important in the context of nematode management, where synthetic nematicides, although effective against target plant-parasitic nematodes (PPN), often exhibit collateral effects on non-target organisms such as free-living nematodes (FLN) and beneficial soil microbes (Qiao *et al*., 2012; Zhang *et al*., 2015).

Free-living nematodes have emerged as sensitive and functionally relevant indicators of soil ecosystem integrity (Neher and Darby, 2009; Du Preez *et al*., 2017). Occupying multiple trophic niches such as bacterivores, fungivores, omnivores, and predators, they serve as bioindicators that reflect nutrient cycling dynamics, organic matter turnover, and ecological stability (Sánchez-Moreno *et al*., 2010; Lazarova *et al*., 2021). Their abundance and community structure respond quickly to changes in land use, cropping intensity, and chemical inputs, making them powerful tools for detecting soil disturbance or recovery (Zhao *et al*., 2024). The Ferris *et al*. (2001) nematode faunal analysis framework, incorporating indices such as the Enrichment Index (EI), Structure Index (SI), Channel Index (CI), and Basal Index (BI) remains a global standard for assessing soil food web condition and functional maturity.

The nematode-based indicators can now be more easily calculated using the nematode indicator joint analysis (NINJA) (Sieriebriennikov *et al*., 2014). Despite their power, nematode-based tools are not without challenges. Accurate assignment of taxa to trophic groups requires expert-level morphological identification or access to molecular tools, both of which are often unavailable in resource-limited settings (van den Hoogen *et al*., 2020). Moreover, nematode extraction and processing are time-consuming and labour-intensive, limiting the scalability of this method for rapid diagnostics.

Complementing nematode indicators, microbial communities, particularly free-living fungi and bacteria, play equally critical roles in soil functioning (Abd-Elgawad, 2020; Poveda *et al*., 2020). They drive organic matter decomposition, nutrient cycling, and pathogen suppression, serving as sensitive indicators of soil recovery or stress (Matos *et al*., 2021; Kasanke *et al*., 2024). Recent innovations such as the microBIOMETER® kit (Prolific Earth Sciences, Montgomery, NY, USA) now allow rapid, field-based assessment of microbial biomass carbon and fungal-to-bacterial ratios. However, limited empirical data exist validating its performance alongside nematode-based soil health indicators under African field conditions.

When used together, FLN-based indices and microbial assays can offer a multi-dimensional view of soil biological responses to management interventions. Yet, no study has jointly applied these complementary tools to assess the ecological impacts of banana fibre paper technology on soil food web structure and microbial balance. Existing evidence shows that chemical nematicides such as fluopyram and abamectin can disrupt non-target soil organisms (Faske and Hurd, 2015; Schleker *et al*., 2022; Qiu *et al*., 2022), whereas biological agents like *Trichoderma spp.* tend to enhance soil microbial activity and diversity (Zhang *et al*., 2015; Asghar *et al*., 2024). However, comparative assessments of these contrasting treatments, when delivered through biodegradable fibre matrices, remain unexplored.

While previous chapters in this thesis evaluated the nematode suppression efficacy and yield benefits of banana fibre paper across different crops, this final chapter shifts attention to the ecological integrity of the soil system itself. This chapter therefore addresses these knowledge gaps by evaluating the effects of fluopyram-, abamectin-, and *Trichoderma*-treated banana fibre papers on nematode faunal indices and soil microbial biomass across potato and pea systems. By integrating nematode-based and microbial indicators, this study provides new evidence on how biodegradable nematicide delivery systems influence soil ecosystem functioning and resilience which is an area critical to the development of climate-smart and biologically integrated nematode management strategies in smallholder farming systems. In sub-Saharan Africa, where smallholder farmers depend heavily on soil natural capital and have limited access to external inputs, pest control methods that also preserve soil biodiversity are not only preferable but also essential.

7.2 Materials and Methods

7.2.1 Experimental design

The experiment was set up similar to section 6.2.1 where potato and pea trials were established at Nyandarua and Nyamira counties of Kenya. This was assessed over two rainy seasons during the short rains in September to November 2022 (Season 1) and long rains in May to July 2023 (Season 2).

7.2.2 Data collection

Free-living nematodes were extracted and quantified similar to plant parasitic nematodes using the modified Baermann's technique (Coyne *et al*., 2014). They were thereafter morphologically identified and classified based on their feeding habits as according to Yeates *et al*. (1993). Four main trophic groups were considered in this study: bacterivores (feeding on bacteria), fungivores (feeding on fungi), predators (feeding on other nematodes) and omnivores (feeding indiscriminately on all soil organisms) (Fig. 7.1).

They were assigned to colonizer-persister (cp) values (Ferris *et al*., 2001). The c-p scale ranges from 1 to 5 and reflects the life-history strategies of nematodes along a colonizer–persister continuum. c-p 1 nematodes (e.g., Rhabditidae) are enrichment opportunists characterized by short life cycles, high fecundity, and rapid response to resource inputs, making them indicators of nutrient enrichment and microbial activity. c-p 2 nematodes represent intermediate colonizers often associated with moderately disturbed soils and nutrient mineralization processes. c-p 3 to c-p 4 groups, including many omnivores and predators, exhibit slower reproduction, longer life cycles, and greater sensitivity to disturbance, thereby serving as indicators of stable, structured soil food webs. c-p 5 nematodes are highly sensitive persisters typically found only in undisturbed, high-quality soils; their presence reflects advanced soil ecological maturity and minimal anthropogenic or chemical stress.

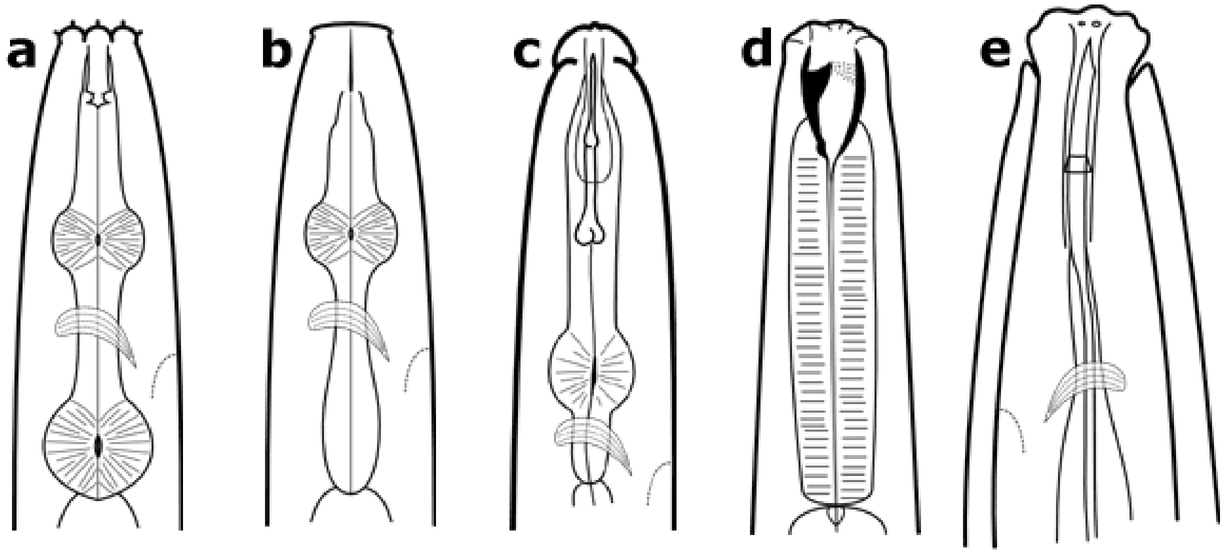

Figure 7.1 Major feeding mouth parts of nematodes: bacterivore (a), fungivore (b), plant-parasitic (c), predator (d) and omnivore (e)

Source: eOrganic (2023)

Assessment of soil microbes was conducted for each crop and site over the two cropping seasons using the microBIOMETER® soil test kit (Fig. 7.2). This entailed collection of soil samples at time of planting from each experimental plot (n = 64) using a hand trowel up to a depth of 20 cm. At harvest time, samples were collected again around the rhizosphere. The microBIOMETER® kit was then used to assess the microbial biomass of fungi and bacteria that were present in the soil before and after application of the treatments, as well as the effect on fungal to bacterial ratio (F:B).

Following the protocol on the microBIOMETER® kit, soils were first sieved to remove large debris. In an extraction tube, 10 ml of distilled water was combined with 10 ml of soil extraction powder and homogenized for 5 seconds using a rotating whisker. A 0.5 ml subsample of soil was then transferred into the extraction tube using a syringe, thoroughly mixed for 30 seconds, and left to rest for 5 minutes. The tube was gently tapped against a hard surface and allowed to settle for an additional 15 minutes to facilitate sediment precipitation and release of microbes into the supernatant (Microbiometer, 2023).

Figure 7.2 microBIOMETER® soil test kit

Source: Microbiometer (2023)

Using a dropper, three drops of the suspension were sequentially applied to a microBIOMETER® test card, whose membrane captures microbes while allowing fluids to drain. Microbial biomass was estimated based on the colour intensity produced on the test card, with images captured via the microBIOMETER® Reader smartphone application and yielded biomass estimates (µg C g^{-1}). To improve accuracy, each sample was analyzed in triplicate.

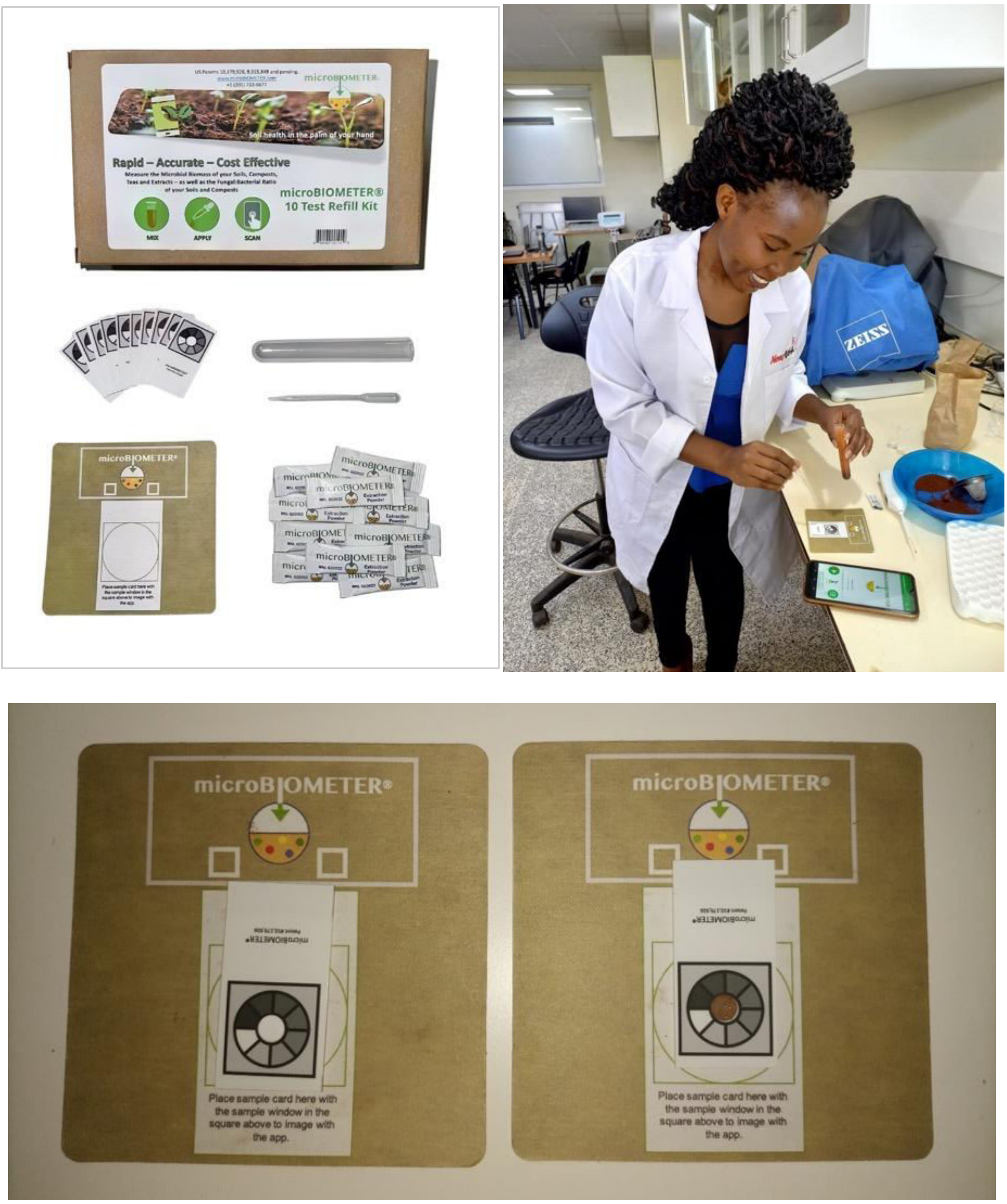

Figure 7.3 Conducting sample test and a test card showing colour gradient through which soil sample is assessed

7.2.3 Data analysis

Nematode ecological indices which are considered as indicators of soil health (maturity index, basal index, enrichment index, structural index, channel index), food web diagnostics and metabolic footprints were calculated online using the nematode indicator joint analysis (NINJA) (Sieriebriennikov *et al.*, 2014). To calculate the percentage variation in microbial biomass, the formulae below was used:

Microbial variation = (Final biomass - Initial biomass)/Initial biomass * 100

Data for free living nematode community structure, FLN abundance, FLN indices, and metabolic footprints were subjected to generalized linear mixed-effects model analysis of variance (ANOVA). Microbial biomass and fungal-to-bacterial (F:B) ratio data were analyzed using a generalized linear model (GLM) to assess the main and interaction effects of treatment and season. Data for each crop trial (potatoes and peas) were presented separately for each site (Nyandarua and Nyamira). Where differences between treatments were observed, the means were separated using Tukey's test at 5% level of probability. All analyses were performed using R version 4.2.3 statistical package (R Core Team, 2023).

7.3 Results

7.3.1 Morphological characterization of free-living nematode assemblages associated with potato and pea agroecosystems

Twenty genera of free-living nematodes assigned to four trophic groups (bacterivores, fungivores, omnivores and predators), were recorded under potato and pea agro-ecosystems throughout the study (Table 7.1). These belonged to 10 families: Aphelenchoidae (*Aphelenchus* spp.), Aphelenchoididae (*Aphelenchoides* spp.), Cephalobidae (*Acrobeles* spp., *Acrobeloides* spp., *Cervidellus* spp., *Cephalobus* spp. and *Eucephalobus* spp.), Dorylaimidae (*Discolaimus* spp., *Dorylaimus* spp. and *Mesodorylaimus* spp.), Mononchidae (*Mylonchulus* spp.), Panagrolaimidae (*Panagrolaimus* spp.), Prismatolaimidae (*Prismatolaimus* spp.), Qudsianematidae (*Eudorylaimus* spp. and *Labronema* spp.), Rhabditidae (*Mesorhabditis* spp., *Osheius* spp. and *Rhabditis* spp.) and Thornematidae (*Prodorylaimus* spp. and *Thornenema* spp.).

All nematode genera were detected in potato trials on both sites whereas only 15 genera were found to be associated with pea agroecosystems (Table 7.1). Among the trophic groups, bacterivore nematodes constituted half of the total genera present in the study with *Rhabditis* being the most dominant genus among all nematodes.

Table 7.1. Percentage contribution of the occurrence, distribution and classification of free-living nematodes in potatoes (cv. Shangi) and peas (cv. Greenfeast) in Nyandarua and Nyamira county, Kenya

		Potatoes		Peas	
Genera	c-p value	Nyandarua	Nyamira	Nyandarua	Nyamira
Bacterivores					
Acrobeles	2	4.57 ± 2.57 c	0.00 ± 0.00 e	6.88 ± 1.53 c	6.50 ± 1.54 d
Acrobeloides	2	0.70 ± 0.59 e	10.91 ± 2.22 c	0.48 ± 0.27 e	9.43 ± 1.68 d
Cervidellus	2	0.00 ± 0.00 e	13.52 ± 1.96 b	0.51 ± 0.51 e	18.03 ± 1.18 b
Cephalobus	2	4.03 ± 1.83 c	0.00 ± 0.00 e	14.13 ± 1.66 a	0.71 ± 0.52 e
Eucephalobus	2	3.00 ± 1.31 d	8.66 ± 2.74 d	1.39 ± 0.51 d	3.60 ± 1.26 e
Mesorhabditis	1	3.02 ± 1.28 d	0.00 ± 0.00 e	1.89 ± 0.91 e	0.00 ± 0.00 e
Osheius	1	0.71 ± 0.71 e	0.30 ± 0.30 e	0.00 ± 0.00 e	0.00 ± 0.00 e
Panagrolaimus	1	0.24 ± 0.24 e	0.00 ± 0.00 e	0.70 ± 0.70 d	0.00 ± 0.00 e
Prismatolaimus	2	4.64 ± 1.83 c	0.00 ± 0.00 e	2.51 ± 0.85 e	1.16 ± 0.67 e
Rhabditis	1	14.12 ± 2.75 a	16.45 ± 3.28 ab	10.93 ± 1.22 b	16.16 ± 1.19 bc
Fungivores					
Aphelenchoides	2	6.48 ± 1.78 b	22.4 ± 2.84 a	15.08 ± 1.37 a	16.96 ± 1.2 b
Aphelenchus	2	17.22 ± 3.95 a	0.72 ± 0.48 e	9.44 ± 1.77 b	0.00 ± 0.00 e
Omnivores					
Dorylaimus	5	9.59 ± 2.41 b	23.25 ± 2.32 a	14.40 ± 1.59 a	25.94 ± 1.63 a
Eudorylaimus	5	5.57 ± 1.77 c	0.00 ± 0.00 e	4.41 ± 1.05 cd	0.00 ± 0.00 e
Labronema	5	2.94 ± 1.31 d	0.00 ± 0.00 e	7.52 ± 1.11 bc	1.2 ± 0.82 e
Mesodorylaimus	5	0.46 ± 0.33 e	0.00 ± 0.00 e	2.37 ± 0.81 e	0.00 ± 0.00 e
Prodorylaimus	5	4.86 ± 1.54 c	0.00 ± 0.00 e	1.86 ± 0.46 e	0.32 ± 0.32 e
Thornenema	5	13.32 ± 3.07 ab	0.00 ± 0.00 e	1.29 ± 2.46 e	0.00 ± 0.00 e
Predators					
Discolaimus	5	2.24 ± 1.41 d	3.78 ± 1.68 d	2.39 ± 0.69 e	0.00 ± 0.00 e
Mylonchulus	5	2.29 ± 1.13 d	0.00 ± 0.00 e	1.83 ± 0.59 e	0.00 ± 0.00 e

c-p value represents life cycle where cp 1 = very short, cp 2 = intermediate, cp 3 and 4 = long, cp 5 = very long
Means represent pooled data for two cropping seasons.
Means followed by the same letters along the columns are not statistically different at $p < 0.05$

Across all field trials, significant differences ($p \leq 0.05$) were observed in the population densities of free-living nematodes (FLN) among the five banana fibre paper treatments evaluated. In potato trials conducted in both Nyandarua and Nyamira, FLN counts varied notably across treatments, with *Trichoderma* paper consistently producing the highest population densities (Table 7.2). In Nyandarua, the FLN abundance under *Trichoderma* paper was 838 nematodes 100 ml^{-1} soil, while in Nyamira the value was similarly high at 851 nematodes 100 ml^{-1} soil. These values were significantly ($p \leq 0.05$) higher than those recorded under any other treatment in the same locations. In Nyandarua, the control had a slightly higher FLN mean than the abamectin paper, whereas in Nyamira, the abamectin paper recorded higher densities than the control. Untreated paper resulted in lower FLN abundance than the control in Nyandarua but exceeded the control values in Nyamira. Fluopyram paper consistently resulted in the lowest FLN densities among all treatments. In both Nyandarua and Nyamira, these plots had markedly reduced FLN numbers, which were significantly lower than those observed in the untreated control plots ($p \leq 0.05$). This trend remained consistent across the entire study period and across locations (Table 7.2).

In the pea trials, treatment effects on FLN densities showed comparable trends to those observed in potato. In both Nyandarua and Nyamira, *Trichoderma* paper recorded the highest FLN populations, followed by the untreated paper and abamectin paper (Table 7.2). In Nyamira, FLN counts under *Trichoderma* paper reached a mean of 483.38 nematodes per 100 ml, while the untreated paper and abamectin paper recorded intermediate values. Fluopyram paper and control exhibited the lowest FLN levels in Nyamira. In Nyandarua, while *Trichoderma* paper again registered the highest FLN counts, statistical analysis revealed no significant differences among treatments ($p > 0.05$). Conversely, treatment differences in Nyamira were statistically significant ($p < 0.001$), with FLN densities consistently varying across treatments.

Table 7.2. Abundance of free-living nematodes among banana fibre paper treatments on potato (cv. Shangi) and pea (cv. Greenfeast) trials in Nyandarua and Nyamira county, Kenya

Trial	Treatment	Nyandarua		Nyamira	
		Mean	SEM	Mean	SEM
Potato	Abamectin	570.25 b	60.97	517.00 b	61.91
	Control	608.75 b	56.39	475.38 b	85.42
	Fluopyram	389.25 c	16.62	364.12 c	40.95
	Trichoderma	838.00 a	64.57	844.50 a	44.67
	Untreated paper	430.25 c	30.20	519.25 b	53.90
Peas	Abamectin	128.31 c	17.76	463.75 c	57.76
	Control	101.31 c	26.03	342.00 d	44.58
	Fluopyram	200.12 bc	67.32	339.00 d	56.70
	Trichoderma	318.56 a	95.91	851.00 a	21.43
	Untreated paper	246.81 ab	67.64	608.25 b	24.19

SEM: Standard error of mean.
Means represent pooled data for two cropping seasons.
Means followed by the same letter(s) along the columns are not statistically different at $p < 0.05$

7.3.2 Effect of banana paper treatments on food web indices and metabolic footprints of free-living nematodes

Except for maturity index (MI), all functional nematode indices varied ($p \leq 0.05$) among the banana paper treatments in potato trials at both sites (Table 7.3). High values of channel (CI) and basal (BI) indices were recorded under plots treated to fluopyram paper in Nyandarua and Nyamira counties. Enrichment (EI) and structural (SI) indices, however, were highest under *Trichoderma* paper and untreated paper plots at both sites.

Table 7.3. Ecological and functional indices of nematodes as affected by banana paper treatments under potato (cv. Shangi) trials at Nyandarua and Nyamira county, Kenya

		Maturity index (MI)	Channel Index (CI)	Basal Index (BI)	Enrichment Index (EI)	Structure Index (SI)
Nyandarua	Abamectin	2.53 ± 0.13	31.55 ± 14.49 b	21.31 ± 4.75 b	57.32 ± 8.38 bc	70.37 ± 6.20 a
	Control	2.50 ± 0.14	30.29 ± 6.41 b	20.16 ± 2.68 b	61.04 ± 2.83 b	69.35 ± 5.18 a
	Fluopyram	2.29 ± 0.18	46.67 ± 9.99 a	32.76 ± 4.16 a	48.79 ± 3.99 c	45.96 ± 11.98 b
	Trichoderma	2.54 ± 0.08	20.94 ± 3.77 c	17.42 ± 2.58 bc	64.82 ± 3.81 ab	74.56 ± 3.63 a
	Untreated	2.46 ± 0.07	11.65 ± 3.29 d	13.86 ± 2.94 c	73.92 ± 4.71 a	77.72 ± 4.48 a
Nyamira	Abamectin	2.38 ± 0.04	27.35 ± 4.36 a	29.47 ± 1.39	47.28 ± 2.76 d	59.74 ± 2.06 b
	Control	2.25 ± 0.02	23.13 ± 4.49 b	24.20 ± 2.31	62.02 ± 4.05 b	60.11 ± 1.93 b
	Fluopyram	2.26 ± 0.03	28.84 ± 3.65 a	39.59 ± 2.94	54.78 ± 2.88 c	24.10 ± 3.98 c
	Trichoderma	2.42 ± 0.03	16.57 ± 2.63 c	21.24 ± 1.99	60.35 ± 3.22 b	78.76 ± 2.32 a
	Untreated	2.24 ± 0.04	12.51 ± 2.06 d	16.07 ± 3.30	70.45 ± 5.23 a	74.69 ± 4.98 a

Means represent pooled data for two cropping seasons.
Within each column, means ± SE followed by different letter(s) indicate significant difference between treatments at $p < 0.05$
Where no letters appear after means, there were no significant differences among the treatments.

Under pea trials, only EI varied significantly among the treatments at both sites (Table 7.4) whereby *Trichoderma* paper recorded the highest EI values relative to the other treatments with 86.57% and 76.13% in Nyandarua and Nyamira, respectively. Untreated paper also recorded significantly ($p < 0.05$) high EI values compared to the control at all sites.

Table 7.4. Ecological and functional indices of nematodes as affected by banana paper treatments under pea (cv. Greenfeast) trials at Nyandarua and Nyamira county, Kenya

		Maturity index (MI)	Channel Index (CI)	Basal Index (BI)	Enrichment Index (EI)	Structure Index (SI)
Nyandarua	Abamectin	2.43 ± 0.23	49.29 ± 22.73	22.78 ± 10.30	62.08 ± 10.47 b	63.59 ± 19.08
	Control	2.55 ± 0.97	50.28 ± 4.35	36.17 ± 24.85	48.48 ± 7.93 c	33.25 ± 47.14
	Fluopyram	2.18 ± 0.21	58.35 ± 3.38	38.27 ± 19.54	55.62 ± 6.07 c	31.07 ± 38.53
	Trichoderma	2.43 ± 0.34	57.85 ± 5.41	34.45 ± 11.61	86.57 ± 4.01 a	44.79 ± 32.09
	Untreated	2.01 ± 0.32	52.24 ± 5.99	32.03 ± 15.62	75.2 ± 8.34 a	29.88 ± 39.77
Nyamira	Abamectin	2.48 ± 0.12	29.5 ± 17.12	27.46 ± 7.01	52.11 ± 6.16 bc	61.95 ± 9.45
	Control	2.41 ± 0.13	20.89 ± 9.52	22.56 ± 4.09	60.80 ± 3.25 b	79.98 ± 16.85
	Fluopyram	2.38 ± 0.07	32.75 ± 10.48	24.26 ± 7.08	43.50 ± 7.07 c	64.83 ± 9.94
	Trichoderma	2.29 ± 0.04	26.09 ± 3.70	24.64 ± 2.55	76.13 ± 7.58 a	60.37 ± 3.66
	Untreated	2.42 ± 0.17	33.28 ± 6.35	32.86 ± 17.44	60.28 ± 10.79 b	54.43 ± 11.51

Means represent pooled data for two cropping seasons.
Within each column, means ± SE followed by different letter(s) indicate significant difference between treatments at $p < 0.05$
Where no letters appear after means, there were no significant differences among the treatments

Metabolic footprints (bacterivore, composite, enrichment, fungivore, omnivore, predator and structural footprints) were statistically different ($p \leq 0.05$) among the banana paper treatments in potato trials at both sites (Table 7.5). Among the trophic groups, the bacterivore footprint was the highest with *Trichoderma* plots recording up to two- and five-fold increase relative to the control and fluopyram plots, respectively, across the sites. Fungivore and predator footprints remained low among the trophic groups in both sites. Enrichment and structure footprint were increased by up to ten-fold under *Trichoderma* plots in Nyandarua and Nyamira.

Table 7.5. Metabolic footprints among banana paper treatments in potato (cv. Shangi) trials at Nyandarua and Nyamira county, Kenya

Site	Metabolic footprint	Abamectin	Control	Fluopyram	*Trichoderma*	Untreated paper
Nyandarua	Bacterivore footprint	688.42 ± 71.34 c	863.56 ± 116.69 b	339.63 ± 28.58 d	1582.37 ± 166.45 a	954.06 ± 65.79 b
	Composite footprint	108.85 ± 34.66 d	147.73 ± 23.15 c	47.95 ± 7.09 e	280.86 ± 46.28 a	219.52 ± 17.86 b
	Enrichment footprint	569.95 ± 70.53 c	698.60 ± 108.89 bc	255.76 ± 32.98 d	1270.54 ± 122.73 a	718.73 ± 62.81 b
	Fungivore footprint	6.80 ± 1.66 cd	13.59 ± 2.84 b	14.39 ± 2.33 b	16.24 ± 0.58 a	4.04 ± 1.82 c
	Omnivore footprint	111.67 ± 36.19 cd	151.37 ± 26.13 c	69.48 ± 7.76 d	295.59 ± 51.53 a	231.29 ± 19.65 b
	Predator footprint	78.32 ± 20.27 b	125.19 ± 10.23 a	131.94 ± 11.41 a	104.18 ± 20.96 ab	28.84 ± 13.19 c
	Structure footprint	491.63 ± 62.28 c	573.41 ± 100.66 bc	123.82 ± 39.76 d	1166.36 ± 132.03 a	689.88 ± 64.60 b
Nyamira	Bacterivore footprint	451.44 ± 48.28 cd	585.16 ± 66.55 c	364.96 ± 53.77 d	1768.33 ± 49.89 a	1444.37 ± 124.62 b
	Composite footprint	75.79 ± 14.24 c	130.71 ± 16.26 b	160.86 ± 19.52 b	297.92 ± 27.05 a	282.48 ± 24.34 a
	Enrichment footprint	339.73 ± 27.69 c	425.41 ± 54.12 c	160.58 ± 39.21 d	1408.64 ± 42.29 a	1133.95 ± 108.44 b
	Fungivore footprint	3.75 ± 0.32 c	5.52 ± 1.05 b	8.84 ± 0.64 a	8.29 ± 0.79 a	5.76 ± 0.81 b
	Omnivore footprint	107.96 ± 20.95 c	154.23 ± 16.96 bc	195.53 ± 29.12 b	351.4 ± 21.90 a	304.65 ± 17.67 a
	Predator footprint	39.61 ± 22.90 a	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b
	Structure footprint	300.13 ± 19.62 b	425.41 ± 54.12 b	160.58 ± 39.21 c	1408.64 ± 42.29 a	1133.95 ± 108.44 a

Means ± standard error represents pooled data from two consecutive cropping seasons.
Across the rows, means followed by different letter(s) indicate significant differences between banana paper treatments at $p < 0.05$

In contrast, no significant differences were detected among banana paper treatments in the pea trials conducted in Nyandarua county ($\chi^2 = 6.53$, $p = 0.42$). However, the results from Nyamira county presented a different pattern, with metabolic footprints showing statistically significant ($\chi^2 = 18.76$, $p < 0.05$) variation across treatments. Notably, the *Trichoderma* paper consistently recorded the highest values for all metabolic footprints, except for the predator footprint, which, while not the highest, remained significantly elevated compared to the control (Table 7.6).

Table 7.6. Metabolic footprints among banana paper treatments in pea (cv. Greenfeast) trials at Nyandarua and Nyamira county, Kenya

Site	Metabolic footprint	Abamectin	Control	Fluopyram	*Trichoderma*	Untreated paper
Nyandarua	Bacterivore footprint	26.49 ± 6.15	11.11 ± 7.03	27.33 ± 12.39	28.11 ± 11.75	36.82 ± 10.96
	Composite footprint	143.49 ± 50.73	50.77 ± 17.95	133.37 ± 47.15	150.06 ± 53.05	90.81 ± 32.11
	Enrichment footprint	28.13 ± 6.02	12.58 ± 5.19	28.93 ± 10.83	33.92 ± 10.13	39.54 ± 4.34
	Fungivore footprint	3.61 ± 1.05	3.93 ± 1.36	4.48 ± 1.43	7.36 ± 3.13	5.78 ± 2.01
	Omnivore footprint	97.01 ± 34.3	30.85 ± 10.91	93.38 ± 33.01	104.39 ± 36.91	39.35 ± 13.91
	Predator footprint	16.38 ± 4.14	4.88 ± 2.22	8.18 ± 4.24	10.20 ± 6.55	8.85 ± 4.34
	Structure footprint	113.39 ± 20.1	35.72 ± 13.04	101.56 ± 58.79	114.59 ± 32.33	48.2 ± 19.41
Nyamira	Bacterivore footprint	36.09 ± 6.47 d	58.26 ± 8.35 c	104.44 ± 10.86 b	194.33 ± 9.46 a	84.61 ± 5.83 bc
	Composite footprint	280.03 ± 52.11 c	287.16 ± 30.64 c	464.83 ± 36.26 d	715.24 ± 36.53 a	411.1 ± 49.09 b
	Enrichment footprint	29.14 ± 6.2 d	57.5 ± 7.75 c	84.94 ± 12.85 b	170.33 ± 8.75 a	62.41 ± 11.19 bc
	Fungivore footprint	3.06 ± 0.56 c	2.11 ± 0.65 d	5.21 ± 1.25 b	9.03 ± 1.21 a	5.78 ± 0.67 b
	Omnivore footprint	240.88 ± 47.88 c	226.79 ± 30.96 c	349.34 ± 27.46 b	497.95 ± 32.86 a	292.29 ± 39.84 c
	Predator footprint	0.00 ± 0.00 d	0.00 ± 0.00 d	5.84 ± 3.99 c	13.93 ± 5.89 b	28.42 ± 10.77 a
	Structure footprint	240.88 ± 47.88 d	226.79 ± 30.96 c	355.19 ± 26.73 b	511.88 ± 31.14 a	320.71 ± 49.82 b

Means ± standard error represents pooled data from two consecutive cropping seasons.
Within each row, means followed by different letter(s) indicate significant difference between banana paper treatments at $p < 0.05$
Where no letters appear after means, there were no significant differences among the treatments.

Food web analysis and metabolic footprints of nematode communities under potato trials indicated variations among the banana paper treatments at both Nyandarua and Nyamira (Fig. 7.4). All treatments resulted in soils that are well-structured and nutrient-enriched except for fluopyram-treated and abamectin-treated plots which led to moderately degraded and disturbed soils, based on food web interpretation scheme (Appendix vi).

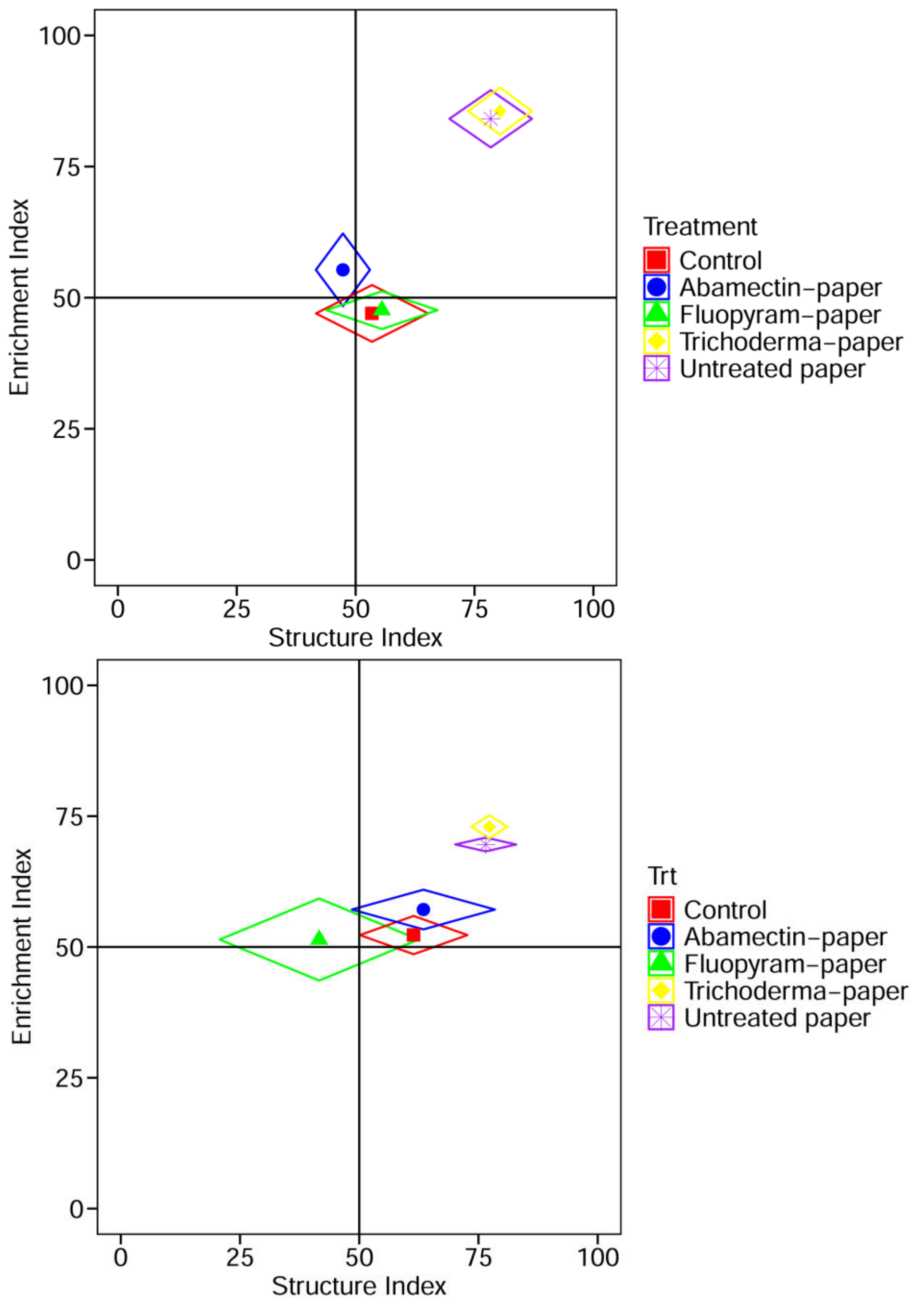

Figure 7.4 Metabolic footprints of nematode communities under potato (cv. Shangi) trials in Nyandarua (top) and Nyamira (bottom) Kenya.

The length of the vertical axis and horizontal axis of the rhombus represents the enrichment and structure footprints, respectively. Graph indicates pooled data from two seasons and two sites.

In pea trials, a similar trend was observed with all plots appearing in the top right quadrant except for fluopyram-treated plots which indicated disturbed but nutrient-enriched, and well-structured soils (Fig. 7.5).

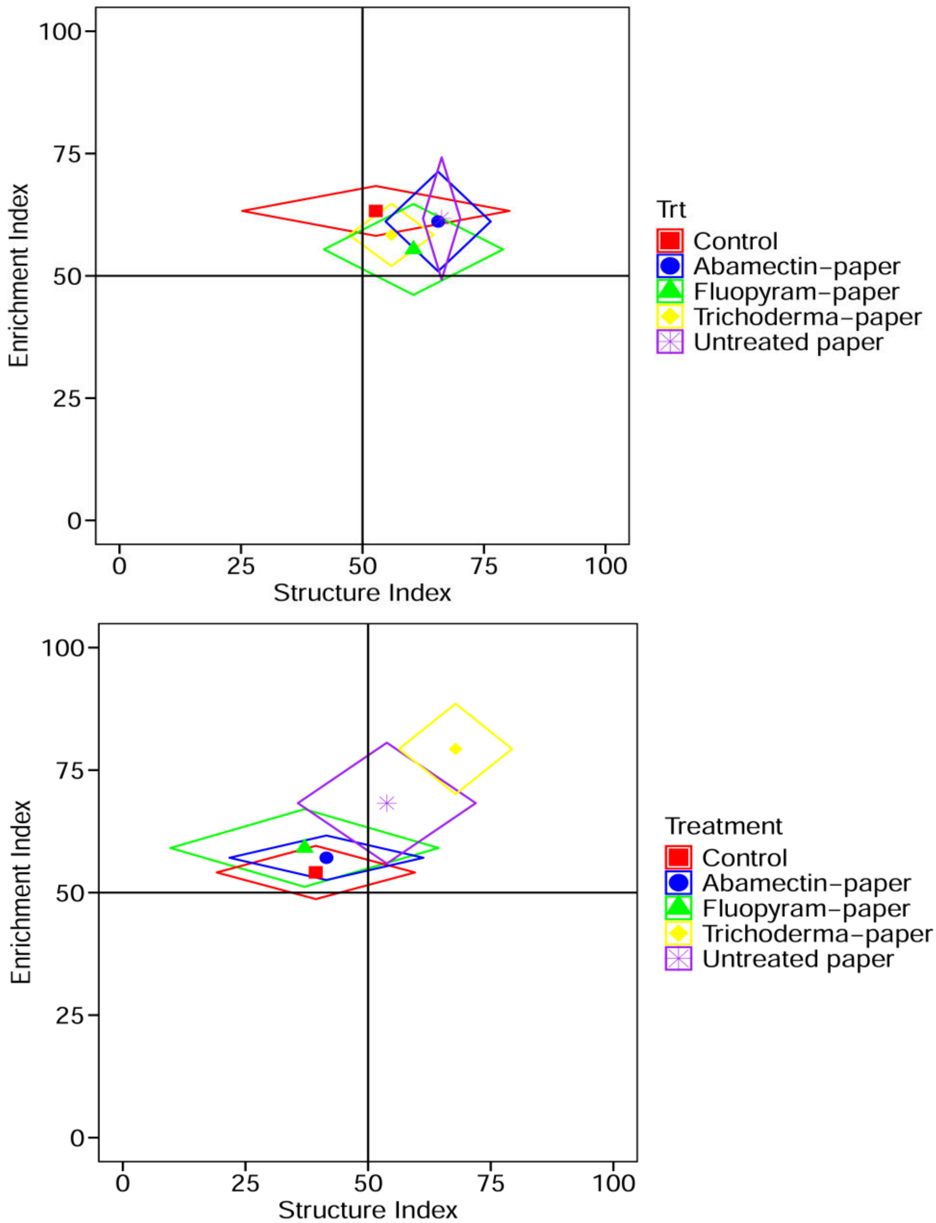

Figure 7.5 Food web analyses of nematode communities under pea (cv. Greenfeast) trials in Nyandarua (top) and Nyamira (bottom), Kenya.

The length of the vertical axis and horizontal axis of the rhombus represents the enrichment and structure footprints, respectively. Graph indicates pooled data from two seasons and two sites.

7.3.3 Microbial buildup among banana paper treatments and sites

There were significant variations in the buildup of fungal and bacterial biomass among the treated banana paper treatments throughout the study (χ^2 = 29.38, p < 0.001). A highly significant effect was also observed for season (χ^2 = 923.97, p < 0.001) and site (χ^2 = 26.06, p < 0.001). Among the interaction terms, the treatment by season interaction was highly significant (χ^2 = 740.66, p < 0.001), indicating that the effect of treatment on microbial abundance varied considerably across seasons. The treatment by site interaction was also significant (χ^2 = 9.63, p = 0.04), suggesting a site-dependent variation in treatment effects.

In potato trials in Nyandarua, plots amended with *Trichoderma*-treated paper and untreated paper showed a higher percentage increase in microbial biomass than abamectin, fluopyram and control plots (Fig. 7.6). This trend was repeated in both cropping seasons with *Trichoderma*-treated paper having the highest values as it recorded a 27.29% increase in season 1 and 98.31% in the second season. On the other hand, and with a similar pattern, potato trials in Nyamira showed both *Trichoderma*-treated paper and untreated paper recording the highest microbial increase during both cropping seasons.

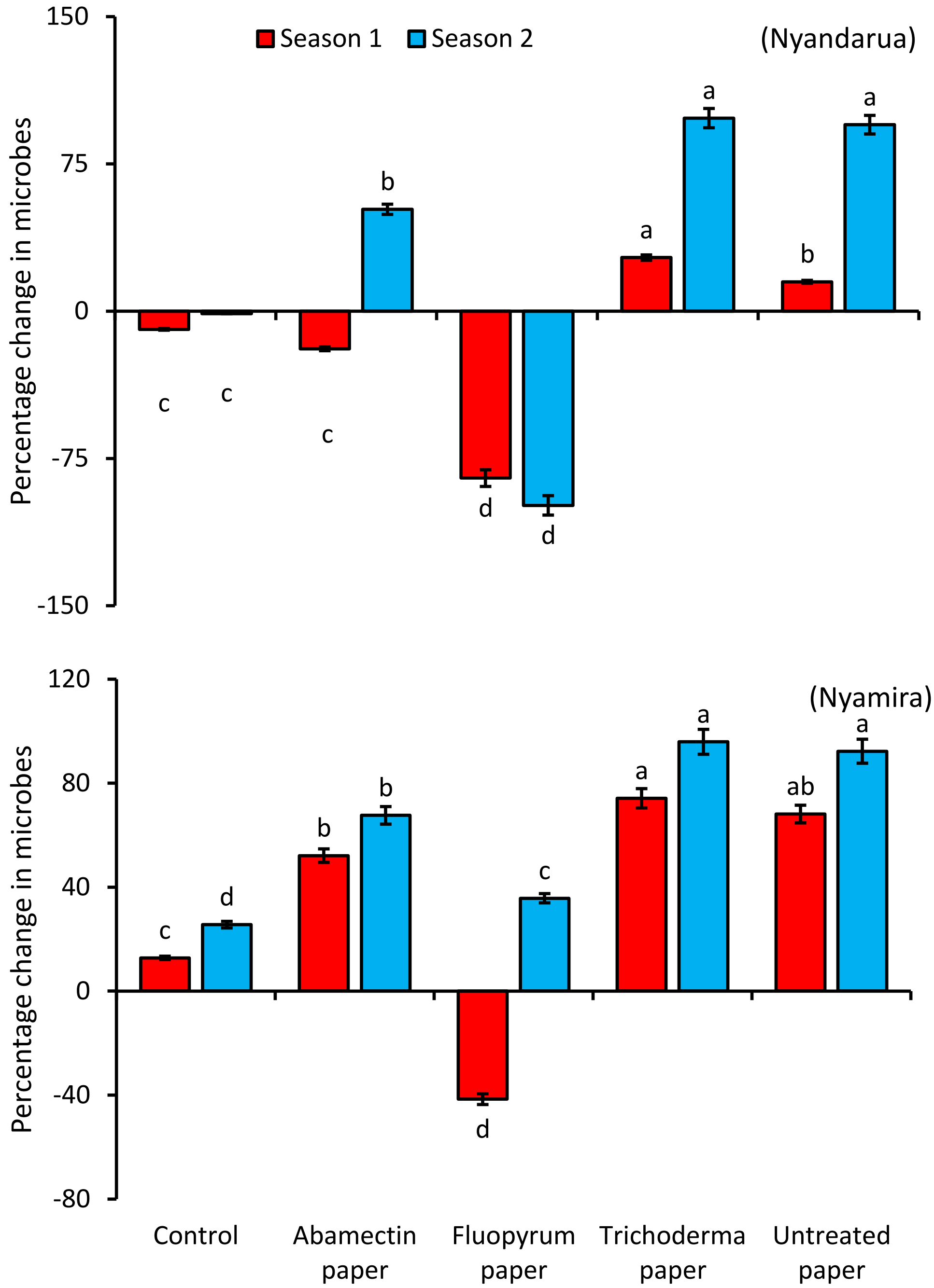

Figure 7.6. Effect of banana paper treatments on the buildup of microbial biomass on two seasons of potatoes (cv. Shangi) at Nyandarua and Nyamira counties of Kenya.

Season 1 = short rains in September to November; Season 2 = long rains in May to July. For each season, bars followed by the same letter are not significantly different at $p < 0.05$.

In the pea trials conducted across Nyandarua and Nyamira counties, plots treated with *Trichoderma*-treated paper and untreated paper exhibited significantly higher microbial biomass compared to the other treatments ($\chi^2 > 8.83$, $p = 0.04$). A statistically significant effect of season was also observed ($\chi^2 > 10.24$, $p = 0.03$), alongside a significant treatment × season interaction ($\chi^2 > 13.21$, $p < 0.001$), indicating that the impact of treatments on microbial biomass varied across cropping seasons. Notably, plots treated with fluopyram- and abamectin-treated banana paper consistently showed reduced microbial biomass in the soil during both seasons at each site (Fig. 7.7).

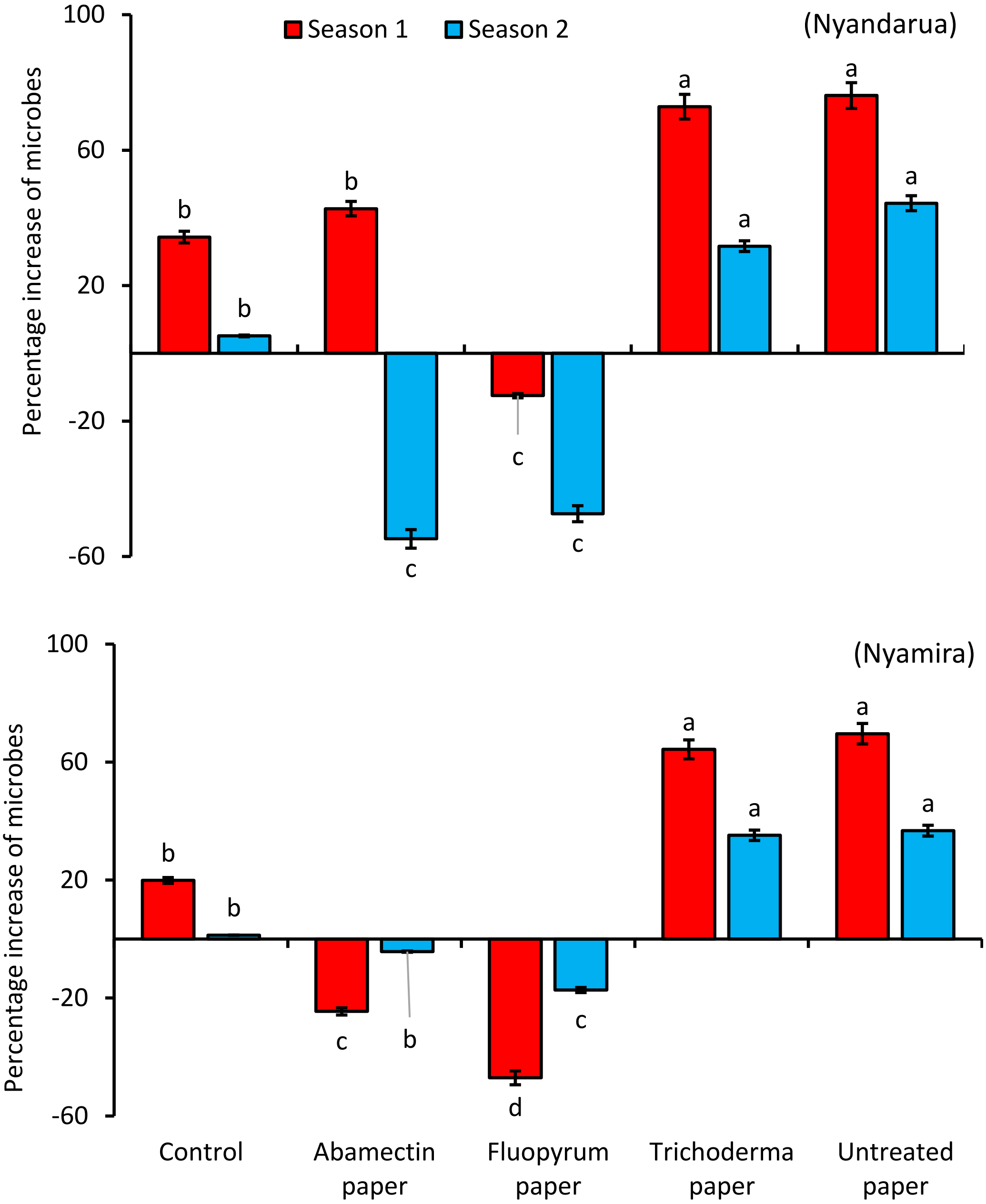

Figure 7.7. Effect of banana paper treatments on the buildup of microbial biomass during two cropping seasons on peas (cv. Greenfeast) at Nyandarua and Nyamira counties of Kenya.

For each season, bars followed by the same letter are not significantly different at $p < 0.05$. Season 1 = short rains in September to November; Season 2 = long rains in May to July.

Fungal-to-bacterial (F:B) ratios varied significantly ($p < 0.05$) across treatments for all crops at each location (Table 7.7). Season effect was not significant and therefore data were pooled together for the two seasons for each trial. In potato, F:B ratios differed significantly in both Nyandarua ($\chi^2 = 18.2$, $p = 0.001$) and Nyamira ($\chi^2 = 24.6$, $p < 0.001$). The highest values were observed in plots treated with *Trichoderma* paper, while the lowest occurred under control and fluopyram paper treatments. In pea, a similar treatment effect was observed at both sites. Significant differences were recorded in Nyandarua ($\chi^2 = 19.3$, $p = 0.001$) and Nyamira ($\chi^2 = 22.1$, $p < 0.001$), with the highest F:B ratios under *Trichoderma* paper, followed by abamectin and untreated paper. The lowest values occurred in control and fluopyram paper treatments. Across both crops and sites, *Trichoderma* paper consistently recorded the highest F:B ratios, while control plots had the lowest.

Table 7.7 Fungal-to-bacterial (F:B) ratios across banana fibre paper treatments under potato (cv. Shangi) and pea (cv. Greenfeast) cropping systems in Nyandarua and Nyamira counties.

	Potatoes		Peas	
	Nyandarua	Nyamira	Nyandarua	Nyamira
Abamectin paper	1.15 ± 0.12 b	1.55 ± 0.09 b	0.66 ± 0.02 b	0.58 ± 0.11 b
Control	0.88 ± 0.07 c	0.54 ± 0.04 c	0.49 ± 0.03 c	0.25 ± 0.04 c
Fluopyram paper	1.36 ± 0.10 b	0.46 ± 0.06 d	0.63 ± 0.02 b	0.31 ± 0.07 c
Trichoderma paper	2.37 ± 0.13 a	2.76 ± 0.14 a	1.88 ± 0.01 a	1.56 ± 0.16 a
Untreated paper	1.15 ± 0.16 b	2.34 ± 0.09 b	0.53 ± 0.20 bc	0.81 ± 0.12 b

Means ± standard error represents pooled data from two cropping seasons.
Within each column, means followed by different letter(s) are significantly different between treatments at $p < 0.05$

7.4 Discussion

The integration of chemically and biologically treated banana fibre paper into the soil environment significantly influenced key indicators of soil health, particularly free-living nematode (FLN) community structure, trophic group composition, nematode-based ecological indices, and soil microbial dynamics. Overall, the results revealed clear functional contrasts between biological and chemical treatments, reflecting how input type determines ecological resilience or disruption within the soil food web.

Trichoderma-treated paper emerged as the most ecologically beneficial amendment, while fluopyram-treated paper consistently exhibited signs of ecological disturbance. This divergence underscores the dual potential of soil-applied technologies to either strengthen or weaken biological networks depending on their mode of action. The increase in FLN diversity and abundance under *Trichoderma* paper aligns with the known capacity of *Trichoderma* spp. to enhance soil biological activity through microbial stimulation and rhizosphere enrichment. This agrees with Zin and Badaluddin (2020), who reported that *Trichoderma* accelerates organic matter degradation, stimulates microbial growth, and improves soil structure. In this study, *Trichoderma* applications promoted proliferation of bacterivore and fungivore nematodes, both critical to nutrient cycling and food web stability. These findings are supported by Hu *et al*. (2015), who observed positive associations between *Trichoderma* amendments and nematode trophic richness in organically managed soils. Additionally, Correa-Delgado *et al*. (2024) documented increased microbial biomass in *Trichoderma*-enriched systems, including boosts in actinomycetes and fungi, further supporting the trends observed here.

Conversely, fluopyram paper consistently recorded declines in FLN abundance and diversity, particularly among higher trophic groups like omnivores and predators. These results demonstrate the trade-offs between chemical efficacy and ecological compatibility. As a succinate dehydrogenase inhibitor (SDHI), fluopyram disrupts mitochondrial respiration not only in target pests but also in non-target soil organisms (Beeman and Tylka, 2018; Schleker *et al*., 2022). Similar patterns have been observed in related studies: Faske and Hurd (2015) reported suppression of beneficial nematodes at even low doses of fluopyram, while Urionabarrenetxea *et al*. (2023) and Lazarova *et al*. (2021) noted trophic downgrading and loss of functional diversity under chemical nematicide stress. The present results therefore corroborate earlier evidence that SDHI-based products, although effective against PPN, can inadvertently reduce biodiversity in the rhizosphere.

Nematode-based soil health indices provided additional insight into these functional shifts. Enrichment Index (EI) and Structure Index (SI), both indicators of trophic complexity and resource quality, were highest under *Trichoderma*-treated and untreated banana paper plots, reflecting biologically enriched and structured ecosystems. These results support findings by Neher (2010) and Sánchez-Moreno and Ferris (2018), who emphasized that biological amendments enhance soil structure and resilience by sustaining trophic diversity. In contrast, Channel Index (CI) and Basal Index (BI) were markedly elevated in fluopyram and abamectin plots. This indicates simplified food webs dominated by stress-tolerant taxa. A high CI reflects bacterial-dominated decomposition pathways (Ferris *et al*., 2001), while elevated BI indicates soil stress and food web degradation (Liu *et al*., 2016; Wang *et al*., 2024). Collectively, these shifts reveal that chemical-based treatments can suppress functional redundancy and destabilize energy flow in soil ecosystems.

Metabolic footprint analysis further emphasized the differential ecological effects of the banana paper treatments. Enrichment and structural footprints, which are indicators of mineralization potential and trophic linkages, were consistently high under *Trichoderma* paper, indicating robust nutrient cycling. These findings align with van den Hoogen *et al*. (2019), who linked high functional footprints to fertile, biologically active soils. In contrast, fluopyram paper exhibited low metabolic footprints across all functional guilds, suggesting suppressed ecosystem functioning. Notably, predator footprints were lowest under fluopyram, supporting the observation by Hu *et al*. (2015) that higher trophic nematodes are most vulnerable to chemical disturbance.

The benefits of *Trichoderma* treatment were evident in both total microbial biomass and fungal-to-bacterial (F:B) ratios, underscoring the strong fungal dominance and decomposition activity facilitated by *Trichoderma* spp. (El Enshasy *et al*., 2020; Poveda *et al*., 2020; Asghar *et al*., 2024). Although a higher F:B ratio is expected under *Trichoderma* enrichment, quantifying this parameter remains essential for assessing the functional balance of the soil microbiome. The F:B ratio serves as a sensitive indicator of the dominant decomposition pathway, with elevated values reflecting enhanced fungal-mediated nutrient cycling, carbon stabilization, and soil organic matter turnover (Strickland and Rousk, 2010; Laine *et al*. 2025). Measuring it under *Trichoderma* treatments thus helps determine whether fungal proliferation translates into functional recovery and structural stability rather than mere biomass accumulation. Furthermore, shifts in the F:B ratio have been associated with soil resilience, aggregation, and long-term organic matter retention (Lehmann *et al*., 2020), making it a relevant indicator for evaluating the broader ecological impacts of biologically based nematode management strategies.

Interestingly, the untreated banana paper also produced relatively high F:B ratios, particularly in potato fields. This could be attributed to its lignocellulosic composition, which provides a substrate for saprophytic fungi involved in decomposing fibrous organic matter (Bhaduri *et al*., 2022). The slow decomposition of untreated paper likely supports fungal energy channels, especially in less-disturbed soils. In contrast, fluopyram plots consistently showed the lowest microbial biomass and F:B ratios across all trials. Since fluopyram is a systemic fungicide, its inhibitory effects on non-target fungi involved in decomposition are well-documented (Zhang *et al*., 2014; Santísima-Trinidad *et al*., 2018). The observed fungal suppression suggests potential long-term risks to soil structure, nutrient cycling, and resilience (Lehmann *et al*., 2020; Matos *et al*., 2021; da Silva *et al*., 2022).

Overall, the findings provide strong evidence that banana fibre paper, particularly when loaded with biological agents like *Trichoderma* spp., can enhance soil biological integrity by maintaining balanced nematode communities and microbial abundance. Conversely, fluopyram paper, consistently reduced biodiversity, altered trophic balance, and disrupted microbial activity. These results bridge the gap between nematode management and soil ecological sustainability, reaffirming the need for integrated approaches that combine efficacy with ecosystem safety. The outcomes reinforce calls by Nicol *et al*. (2011), and Sikora and Roberts (2018) for nematode management strategies that embed biological compatibility within chemical control frameworks to sustain long-term soil health.

CHAPTER EIGHT: GENERAL CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

This study addressed the pressing challenge of plant parasitic nematodes (PPN) in potato- and pea-based cropping systems by evaluating banana fibre paper as a biodegradable carrier for chemical (fluopyram, abamectin) and biological (*Trichoderma* spp.) nematicides. Through multi-season field trials across contrasting agroecological zones, the research assessed treatment effects on nematode suppression, crop yield, and soil health indicators, including free-living nematodes and microbial biomass. The results reveal critical interactions between banana paper treatments, crop cultivar, and ecological responses, thereby contributing significantly to integrated nematode management strategies.

8.1 Conclusions

The conclusions drawn from this study are:

i. Both abamectin- and fluopyram-treated banana fibre papers effectively suppressed PPN densities and improved potato yields. Fluopyram-paper was particularly effective under high nematode pressure, especially in susceptible cultivars. This demonstrates the potential of banana paper as a localized delivery system for nematicides for enhanced nematode management.

ii. Fluopyram-treated paper performed comparably to direct fluopyram application in suppressing nematodes and boosting yield, particularly in the susceptible cultivar Shangi. The comparable performance of paper-incorporated fluopyram suggests that biodegradable carrier systems can reduce chemical inputs while maintaining efficacy, offering a more economic nematode management strategy for susceptible potato cultivars

iii. While banana fibre paper treatments improved nematode management and yield in potato-based systems, effects in peas were less pronounced. The limited efficacy observed in peas likely resulted from the smaller seed size, reduced paper-to-seed contact, and low nematode baseline populations. These findings indicate that the wrapping technology is more suited to large-seeded or tuber crops and that further adaptation is required for small-grain legumes.

iv. Banana fibre paper treatments significantly influenced soil health. *Trichoderma*-treated paper improved microbial diversity and biomass, promoting soil resilience. In contrast, fluopyram-treated plots showed signs of disturbance to the microbial equilibrium. This implies that biological options are more suitable for long-term soil sustainability.

8.2 Recommendations

Based on the findings of this study, several recommendations are proposed to guide future nematode management practices, research priorities, and policy directions in smallholder farming systems:

i. Farmers should adopt banana fibre paper impregnated with effective nematicides, such as fluopyram, to achieve targeted nematode suppression in potatoes, particularly in high-pressure fields, enhancing yield while minimizing environmental contamination. Having been recently banned in Kenya, abamectin products should be avoided.

ii. Biodegradable fluopyram paper should be recommended as an economical alternative to conventional chemical application, particularly for susceptible cultivars like Shangi, allowing smallholders to reduce chemical input while still maintaining nematode control efficacy.

iii. For small-seeded legumes like peas, the banana fibre technology should be adapted, such as through seed coatings or pellets, to improve uptake, contact, and nematode management effectiveness in crops other than potatoes and/or tuberous crops.

iv. Biological amendments, particularly *Trichoderma*-treated banana paper, should be prioritized in ecologically sensitive or low-input organic systems to restore and maintain soil biodiversity, structure, and long-term productivity. Its use should be scaled as part of regenerative agriculture programs targeting long-term soil fertility and resilience.

8.3 Suggestions for further research

i. There is a need for extended in-depth assessments to examine the cumulative ecological effects of banana paper treatments on soil biodiversity and productivity.

ii. Molecular techniques should be employed to unravel how treatments alter microbial community structure and root-soil interactions at the genus level.

iii. A comprehensive cost-benefit analysis comparing banana paper to conventional nematicide application methods is essential to support large-scale uptake.

iv. Further trials are needed to refine dosages and combinations of biological agents that can be integrated into the banana paper matrix.

v. Evaluate the banana fibre paper technology on disease complexes such as nematode-bacteria or nematode-fungus interactions.

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APPENDICES

Appendix i: Proposal approval

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY (MMUST)

Tel: 056-30870
Fax: 056-30153
E-mail: directordps@mmust.ac.ke
Website: www.mmust.ac.ke

P.O Box 190
Kakamega — 50100
Kenya

Directorate of Postgraduate Studies

Ref: MMU/COR: 509099

9th November 2022

Janet G. Atandi
SCP/H/01-54980/2020
P.O. Box 190-50100
KAKAMEGA

Dear Ms. Atandi,

RE: APPROVAL OF PROPOSAL

I am pleased to inform you that the Directorate of Postgraduate Studies has considered and approved your PhD proposal entitled: ***'Implication of using biodegradable Banana Fibre Paper in Nematode Management and Soil Health in Kenya'*** and appointed the following as supervisors:

1. Dr. Dennis Omayio - MMUST
2. Dr. Kanan Saikai - MMUST
3. Prof. Danny L. Coyne - MMUST

You are required to submit through your supervisor(s) progress reports every three months to the Director of Postgraduate Studies. Such reports should be copied to the following: Chairman, School of Natural Sciences Graduate Studies Committee; Chairman, Department of Biological Sciences & Departmental Graduate Studies Committee. Kindly adhere to research ethics consideration in conducting research.

It is the policy and regulations of the University that you observe a deadline of two years from the date of registration to complete your PhD thesis. Do not hesitate to consult this office in case of any problem encountered in the course of your work.

We wish you the best in your research and hope the study will make original contribution to knowledge.

Yours sincerely,

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY DIRECTORATE OF POSTGRADUATE STUDIES P.O. Box 190 - 50100, KAKAMEGA (K)

Prof. Stephen O. Odebero, PhD, FIEEP
DIRECTOR, DIRECTORATE OF POSTGRADUATE STUDIES

Appendix ii: Research permit from University ISERC

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY

Tel: 056-31375 | P. O. Box 190,
Fax: 056-30153 | 50100.
E-mail: ierc@mmust.ac.ke | Kakamega,
Website: www.mmust.ac.ke | **KENYA**

Institutional Scientific and Ethics Review Committee

REF: MMU/COR: 40312 Vol 6(01) | Date: July 14th, 2025

To: Ms. Janet G. Atandi

Dear Ms. Atandi,

RE: LONG-TERM IMPLICATIONS OF USING BIODEGRADABLE BANANA FIBRE PAPER AS AN ORGANIC CARRIER IN THE MANAGEMENT OF NEMATODES AND SOIL HEALTH.

This is to inform you that the ***Masinde Muliro University of Science and Technology Institutional Scientific and Ethics Review Committee (MMUST-ISERC)*** has reviewed and approved your above research proposal. Your application approval number is **MMUST/ ISERC/129/2025.** The approval covers for the period ***July 14th, 2025** to **July 14tt, 2026.***

This approval is subject to compliance with the following requirements;

i. Only approved documents including informed consents, study instruments, MTA will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***MMUST-ISERC.***
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***MMUST-ISERC*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***MMUST-ISERC*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to ***MMUST-ISERC.***

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed

Yours Sincerely,

Prof. Gordon Nguka (PhD)
Chairperson, Institutional Scientific and Ethics Review Committee

Copy to:
- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)

Appendix iii: Research Certificate from NACOSTI

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **850889**

Date of Issue: **21/June/2025**

RESEARCH LICENSE

This is to Certify that Ms.. Janet G. Atandi of Masinde Muliro University of Science and Technology, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Nyamira, Nyandarua on the topic: Impact of modified banana paper technology on plant-parasitic nematodes and soil health for the period ending : 21/June/2026.

License No: **NACOSTI/P/25/4175502**

850889

Applicant Identification Number

Deputy Director

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

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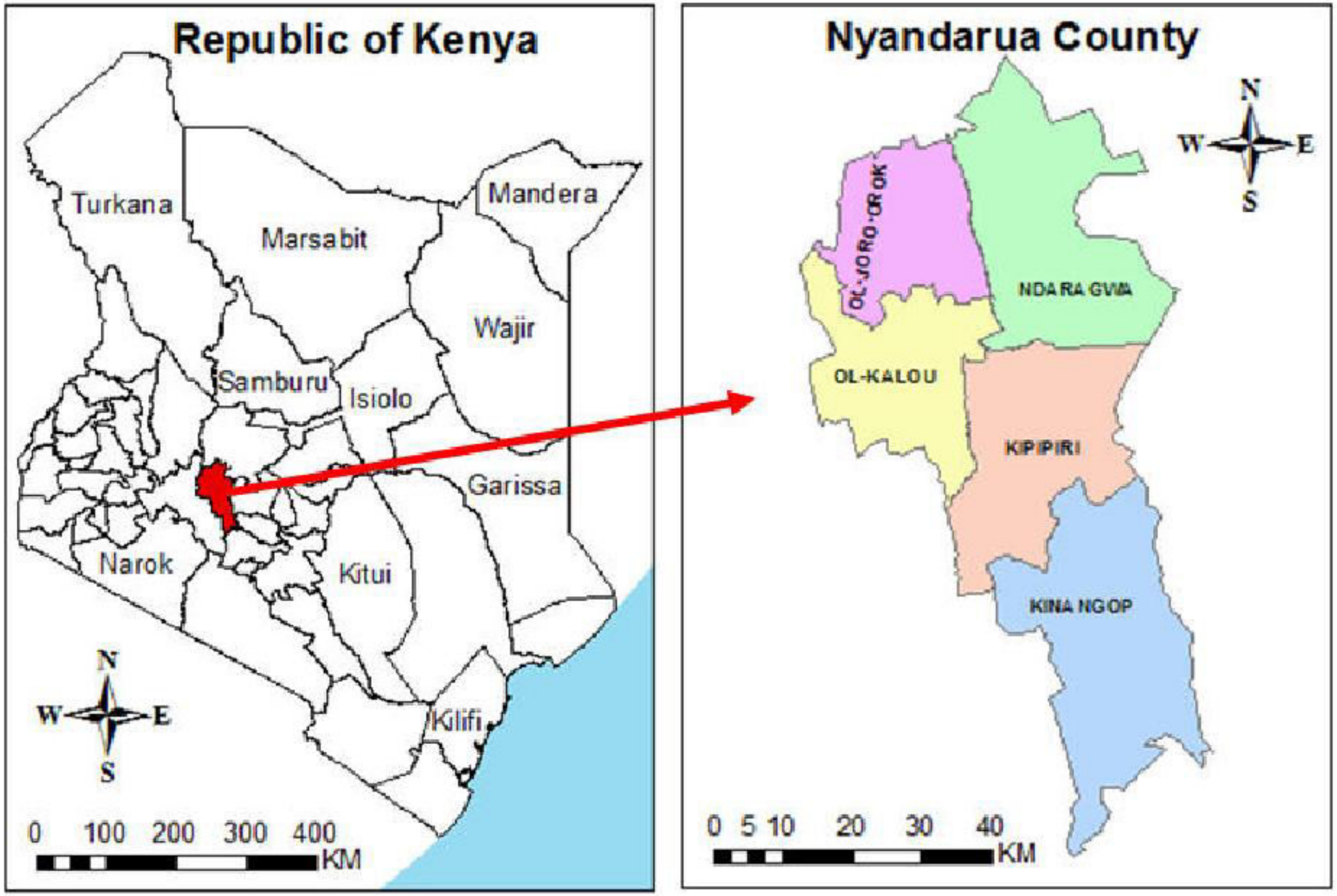
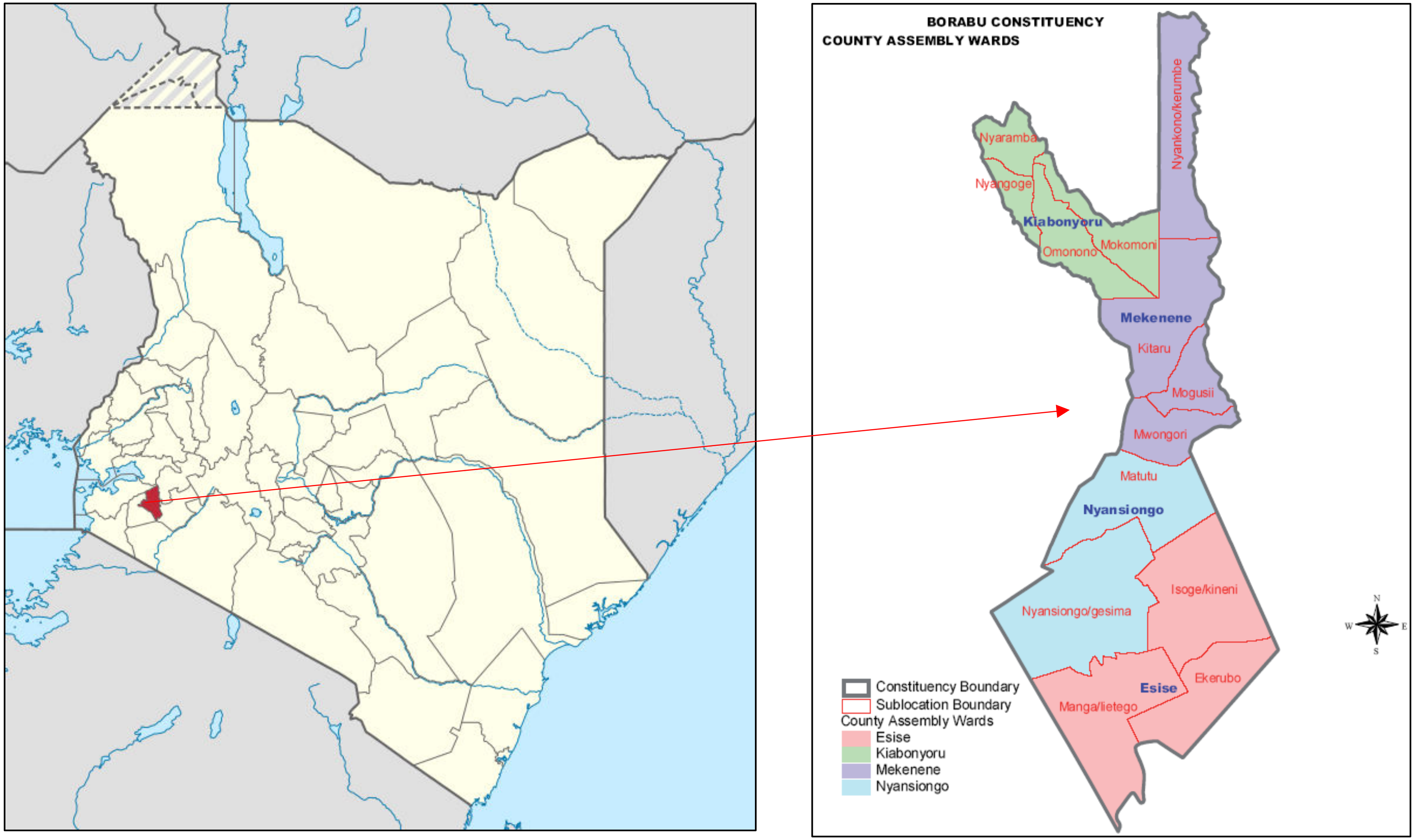
See overleaf for conditions

Appendix iv: Map of Nyandarua county, Kenya



Appendix v: Map of Nyamira county, Kenya



Appendix vi: Average rainfall and temperature during the study period

	Objective	Season	Period	Rainfall (mm)	Mean temperature
Trial #1 Nyandarua	Objective 1	Season 1	Mar - May '21	578.67	17.23˚C
		Season 2	Nov '21 - Jan '22	357.23	17.10˚C
Trial #2 Nyandarua	Objective 1	Season 1	Mar - May '21	578.67	17.23˚C
		Season 2	Jul - Sep '21	169.85	16.11˚C
		Season 3	Nov '21 - Jan '22	357.23	17.1˚C
Trial #3 Nyandarua	Objective 2	Season 1	Mar - May '22	428.15	18.02˚C
		Season 2	Jul - Sep '22	437.12	15.79˚C
Trial #4 Nyandarua	Objective 3 & 4	Season 1	Nov '22 - Jan '23	590.73	16.27˚C
		Season 2	Mar - May '23	533.83	17.30˚C
		Season 3	Jul - Sep '23	134.7	16.64˚C
		Season 4	Nov '23 - Jan '24	510.75	16.73˚C
Trial #4 Nyamira	Objective 3 & 4	Season 1	Mar - May '23	859.76	18.37˚C
		Season 2	Jul - Sep '23	636.93	17.30˚C
		Season 3	Nov '23 - Jan '24	372.06	17.17˚C

Rainfall represents daily rainfall collected over the duration
Temperature is the mean calculated between highest and lowest daily temperatures averaged per season.

Appendix vii: Morphological identification of key plant parasitic nematodes

Genus	Body length	Stylet (type and approximate length)	Key distinguishing morphological features	Reference
Globodera (cyst nematode)	~445–510 µm (juveniles) in cyst species.	Stomatostylet ~18–29 µm with basal knobs.	Robust J2s, hyaline tail region, cyst body females or eggs in cyst, strong stylet knobs.	PM 7/40 (EPPO) 2022 (EPPO Global Database)
Meloidogyne (root-knot nematode)	J2 ~350-450 µm in some reports; females longer.	Stomatostylet ~12-16 µm in J2s; ~15-18 µm in other life-stages.	Females swollen/pear-shaped, perineal pattern in root-knots, juveniles slender with short stylet.	Perry & Moens, 1998; other morphological keys.
Trichodorus (stubby-root/virus-vector nematode)	~0.8-1.1 mm (800-1100 µm) in some species.	Onchiostyle (rather than a typical hollow stylet) ~47-59 µm (for *T. marylandi* as example).	Ectoparasitic, onchiostyle curved, short stubby-rooted damage, virus vector potential.	UGhent, 2024
Pratylenchus (lesion nematode)	~400-600 µm in many species; e.g., ~670 µm reported.	Stomatostylet/odontostylet ~16-20 µm in many species.	Migratory endoparasite, cylindrical body, four lateral incisures typical, tail slender, lip region moderate.	KSU, 2023.
Helicotylenchus (spiral nematode)	~500-800 µm in many populations.	Stomatostylet ~22-24 µm reported in one figure.	Body coiled or spiral when relaxed, lips truncated ringed, tail shape variable, lateral field typically two or four lines.	KSU, 2023.

Appendix viii: ANOVA results

Three-way ANOVA for nematode genera

Season	LR	Chisq	Df	Pr(>Chisq)	
1	Treatment	1.9	1	0.1631	
	Variety	0.0	1	0.96196	
	Genus	184.3	12	2.00E-16	***
	Treatment: Variety	0.0	1	0.87417	
	Treatment: Genus	8.8	12	0.7205	
	Variety: Genus	21.6	12	0.04218	*
	Treatment: Variety: Genus	1.1	12	0.99998	
2	Variety	0.2	1	0.6586	
	Treatment	63.9	1	1.34E-15	***
	Genus	45.2	12	9.44E-06	***
	Variety: Treatment	0.0	1	0.9317	
	Variety: Genus	1.0	12	1	
	Treatment: Genus	4.5	12	0.9725	
	Variety: Treatment: Genus	8.8	12	0.7215	

Three-way ANOVA for yield

	LR	Chisq	Df	Pr(>Chisq)	
Total yield	Variety	14.3	1	0.0001569	***
	Treatment	24.7	1	6.56E-07	***
	Season	3.0	1	0.08119	
	Variety: Treatment	0.1	1	0.772385	
	Variety: Season	4.9	1	0.0273624	*
	Treatment: Season	1.1	1	0.2932187	
	Variety: Treatment: Season	0.3	1	0.5837007	
Marketable yield	Variety	0.2	1	0.68102	
	Treatment	3.6	1	0.05753	.
	Season	1.6	1	0.20240	
	Variety: Treatment	0.0	1	0.95742	
	Variety: Season	0.1	1	0.76448	
	Treatment: Season	0.1	1	0.74914	
	Variety: Treatment: Season	0.1	1	0.76618	

Two-way ANOVA for cyst populations

Cysts	LR	Chisq	Df	Pr(>Chisq)	
Initial population	Season	7.6331	2	0.022	*
	Treatment	0.0061	1	0.9377	
	Season: Treatment	0.0188	2	0.9906	
Final population	Season	36.5	2	1.16E-08	***
	Treatment	59.3	1	1.35E-14	***
	Season: Treatment	3.5	2	0.176	
Reproductive factor	Season	14.3	2	0.0007849	***
	Treatment	13.202	1	0.0002796	***
	Season: Treatment	1.427	2	0.4899165	

Three-way ANOVA for nematode genera

LR	Chisq	Df	Pr(>Chisq)	
Season	146.234	1	2.20E-16	***
Variety	7.697	1	0.005531	**
Treatment	7.847	4	0.097329	.
Genus	188.68	11	2.20E-16	***
Season: Variety	7.333	1	0.006771	**
Season: Treatment	6.555	4	0.161329	
Variety: Treatment	2.135	4	0.710984	
Season: Genus	191.085	11	2.20E-16	***
Variety: Genus	11.421	11	0.408732	
Treatment: Genus	50.957	44	0.218856	
Season: Variety: Treatment	2.231	4	0.69333	
Season: Variety: Genus	11.207	11	0.426124	
Season: Treatment: Genus	49.522	44	0.262357	
Variety: Treatment: Genus	29.581	44	0.952893	
Season: Variety: Treatment: Genus	29.622	44	0.952327	

Three-way ANOVA for cyst nematodes

LR	Chisq	Df	Pr(>Chisq)	
Season	5.6922	1	0.017041	*
Variety	4.2494	1	0.039264	*
Treatment	27.6426	4	1.47E-05	***
Season: Variety	7.4936	1	0.006192	**
Season: Treatment	2.1331	4	0.711296	
Variety: Treatment	1.9465	4	0.745598	
Season: Variety: Treatment	6.5476	4	0.161813	

Two-way ANOVA for yield

Yield	LR	Chisq	Df	Pr(>Chisq)	
Total	Treatment	14.572	1	0.0001349	***
	Season	2.6314	2	0.2682832	
	Treatment: Season	1.6162	2	0.4457109	
Marketable	Treatment	15.635	1	7.68E-05	***
	Season	4.208	2	0.122	
	Treatment: Season	0.6739	2	0.7139	
Unmarketable	Treatment	0.10588	1	0.7449	
	Season	2.66235	2	0.2642	
	Treatment: Season	1.31013	2	0.5194	

Three-way ANOVA for nematodes

Nematode	LR	Chisq	Df	Pr(>Chisq)	
Abundance	Season	508.5	1	2.20E-16	***
	Variety	90.0	1	2.20E-16	***
	Treatment	65.9	4	1.65E-13	***
	Season: Variety	92.0	1	2.20E-16	***
	Season: Treatment	64.2	4	3.86E-13	***
	Variety: Treatment	61.8	4	1.24E-12	***
	Season: Variety: Treatment	59.8	4	3.23E-12	***
Genera	Variety	0.5	1	0.495159	
	Treatment	10.5	4	0.032652	*
	Genus	169.1	11	2.20E-16	***
	Variety: Treatment	2.9	4	0.579279	
	Variety: Genus	12.4	11	0.332417	
	Treatment: Genus	71.8	44	0.005097	**
	Variety: Treatment: Genus	63.9	44	0.026639	*

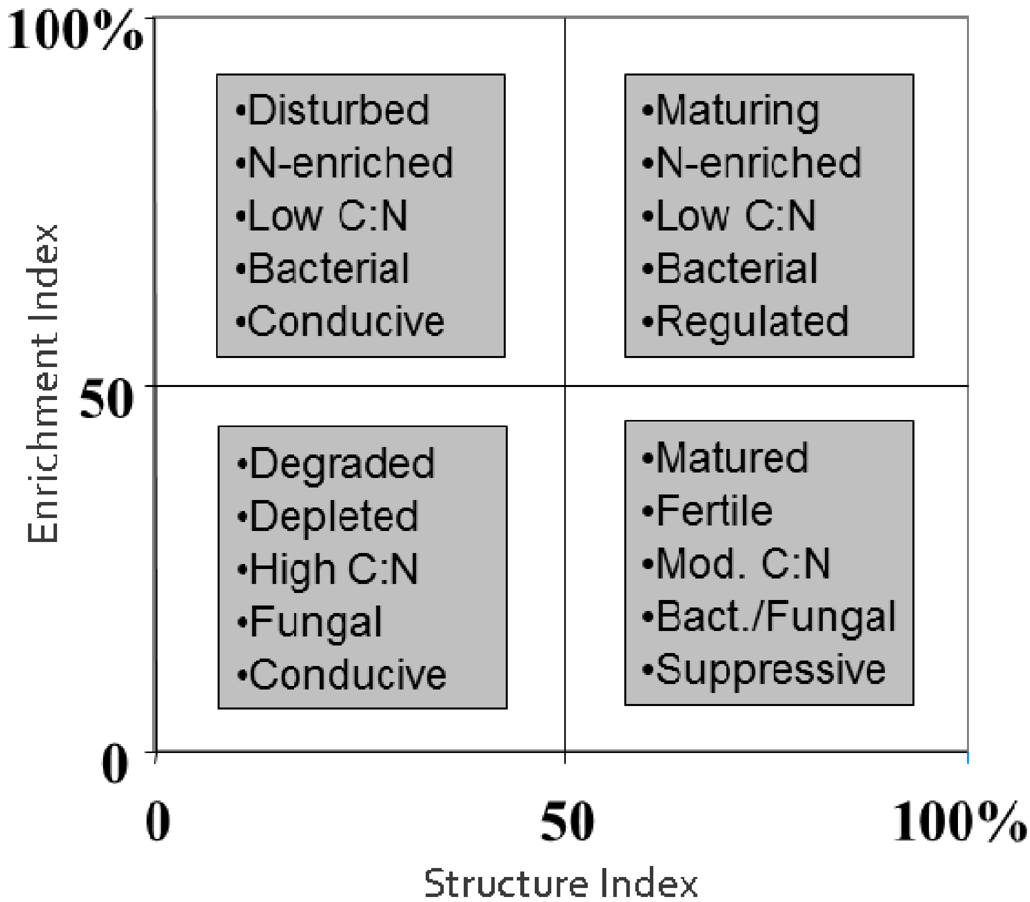
Three-way ANOVA for yield

	LR	Chisq	Df	Pr(>Chisq)	
Total yield	Season	477.1	3	2.20E-16	***
	Site	1188.1	1	2.20E-16	***
	Treatment	31.3	4	2.69E-06	***
	Season: Site	186.6	3	2.20E-16	***
	Season: Treatment	52.1	12	5.94E-07	***
	Site: Treatment	2.8	4	0.586676	
	Season: Site: Treatment	26.4	12	0.009301	**
Marketable yield	Season	117.5	1	2.20E-16	***
	Site	611.1	1	2.20E-16	***
	Treatment	18.5	4	0.0009983	***
	Season: Site	26.9	1	2.20E-07	***
	Season: Treatment	13.2	4	0.0104999	*
	Site: Treatment	3.6	4	0.4577085	
	Season: Site: Treatment	2.3	4	0.6870137	
Unmarketable yield	Season	184.4	1	<2e-16	***
	Site	173.608	1	<2e-16	***
	Treatment	4.906	4	0.297	
	Season: Site	162.812	1	<2e-16	***
	Season: Treatment	0.932	4	0.9199	
	Site: Treatment	5.495	4	0.2402	
	Season: Site: Treatment	0.509	4	0.9726	

Three-way ANOVA for microbes

Microbial biomass	LR	Chisq	Df	Pr(>Chisq)	
Potatoes	Treatment	29.38	4	6.55E-06	***
	Season	923.97	2	2.20E-16	***
	Site	26.06	1	3.32E-07	***
	Treatment: Season	740.66	8	2.20E-16	***
	Treatment: Site	9.63	4	0.04723	*
	Season: Site	0.465	1	0.495159	
	Treatment: Season: Site	13.396	4	0.009496	**
Peas	Treatment	15.027	4	0.004646	**
	Season	6.078	1	0.013687	*
	Site	105.032	1	2.20E-16	***
	Treatment: Season	1.579	4	0.812542	
	Treatment: Site	3.714	4	0.446078	
	Season: Site	15.85	1	6.86E-05	***
	Treatment: Season: Site	25.902	4	3.31E-05	***

Appendix ix: Interpretation scheme for food web analysis of nematode communities



Appendix x: Publications

RESEARCH NOTE

BANANA FIBRE PAPER TECHNOLOGY: IMPACT OF REPEATED USE ON PLANT-PARASITIC NEMATODES AND YIELD OF POTATOES AND PEAS

Janet G. Atandi[1,2]*, Calvince Orage[2], Eva I. Nyambura[2], Agnes W. Kiriga[3], Dennis O. Omayio[1], Kanan Saikai[2], Solveig Haukeland[3,4], James Kisaakye[2] and Danny L. Coyne[2]*

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ABSTRACT

Atandi, J. G., C. Orage, E. I. Nyambura, A. W. Kiriga, D. O. Omayio, K. Saikai, S. Haukeland, J. Kisaakye and D. L. Coyne. 2025. Banana fibre paper technology: Impact of repeated use on plant-parasitic nematodes and yield of potatoes and peas. Nematropica 55:?-?

Crop losses from plant-parasitic nematodes in sub-Saharan Africa necessitate sustainable, affordable, and smallholder-adapted management options. Synthetic nematicides are effective but difficult to apply safely. Using banana fibre paper (BFP) as a biodegradable carrier for low dose nematicide delivery offers a promising alternative. Our study tested the approach through consecutive potato (*Solanum tuberosum*) and pea (*Pisum sativum*) cropping at two locations in Kenya. Banana paper delivered biological (*Trichoderma asperellum*) and chemical (abamectin, fluopyram) nematicides. Nineteen nematode genera were recovered, including *Globodera*, *Trichodorus*, and *Meloidogyne*. Better nematode suppression was observed in the BFP + Abamectin, BFP + Fluopyram and BFP + *Trichoderma* treatments, relative to the control, albeit inconsistently. While BFP + Fluopyram and BFP + *Trichoderma* increased potato yields compared to the control, pea yields remained unchanged. Banana fibre paper technology presents an effective option for pesticide delivery, minimizing excessive use and potential contamination. The findings also offer a basis for training farmers in safe pesticide practices for nematode control.

Key words: abamectin; fluopyram; nematicides; *Trichoderma*

RESUMEN

Atandi, J. G., C. Orage, E. I. Nyambura, A. W. Kiriga, D. O. Omayio, K. Saikai, S. Haukeland, J. Kisaakye and D. L. Coyne. 2025. Banana fibre paper technology: Impact of repeated use on plant-parasitic nematodes and yield of potatoes and peas. Nematropica 55:?-?

Las pérdidas de cultivos causadas por nematodos fitoparásitos en África subsahariana exigen opciones de manejo sostenibles, asequibles y adaptadas a los pequeños agricultores. Los nematicidas sintéticos son eficaces, pero su aplicación segura resulta difícil. El uso de papel de fibra de plátano como portador biodegradable para la aplicación de bajas dosis de nematicidas ofrece una alternativa prometedora. Nuestro

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POTENTIAL FOR BANANA FIBRE PAPER FOR CONTROLLING NEMATODE PESTS IN POTATO PRODUCTION IN KENYA

POTENTIAL FOR BANANA FIBRE PAPER FOR CONTROLLING NEMATODE PESTS IN POTATO PRODUCTION IN KENYA

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ABSTRACT

Potato (*Solanum tuberosum*) is an increasingly important staple in sub-Saharan Africa (SSA), driven by rapid urbanisation and rising demand for fast foods. Unfortunately, production of the crop is marred by a number of constraints, one of which is plant-parasitic nematodes (PPN) such as the *Globodera* spp., commonly known as the potato cyst nematode (PCN). Existing means of nematode control present major challenges such as pesticide contamination; while many products have been withdrawn from the market due to environmental toxicity allegations. The objective of this study was to evaluate the efficacy of banana fibre paper impregnated with low dosages of the nematicides abamectin and fluopyram for nematode management in potato production in Kenya. A field study was conducted at Kinangop, Nyandarua County during short and long rains in 2020 and 2021, respectively. Treatments included abamectin-treated paper, fluopyram-treated paper, untreated paper and direct fluopyram application alongside a control. Abamectin-treated paper reduced nematode populations by 28.6% relative to the control; while the fluopyram-treated counterpart achieved up to 66.8% suppression; and demonstrated the strongest overall performance. Potato tuber yields were enhanced, respectively, by 3.1 and 5.9 t ha^{-1}, by fluopyram-based treatments. Hence, banana fibre paper offers a promising alternative to conventional nematicide application by delivering lower chemical doses more precisely aligning well with smallholder production systems. These results demonstrate that banana