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Nematology 27 (2025) 25-36

Nematology
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Efficacy of banana fibre paper for the management of the root-knot nematode, *Meloidogyne incognita*, on potato (*Solanum tuberosum*) in Kenya

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Received: 6 September 2024; revised: 12 October 2024
Accepted for publication: 16 October 2024; published online: 22 November 2024

Summary – Globally, potato (*Solanum tuberosum*) is a key staple food crop. In Kenya, it is the second most important food crop after maize. Among the various constraints to potato production are plant-parasitic nematodes. In particular, root-knot nematodes (*Meloidogyne* spp.) are a significant impediment to potato production, suppressing yield and reducing the quality of harvested tubers. The current study was undertaken to evaluate the efficacy of a lignocellulose fibre matrix (banana paper) either impregnated with a chemical or drenched with a biologically-based nematicide for the management of root-knot nematodes on potatoes, in Kenya. The experiment was conducted in both field and pot trials over two consecutive cropping seasons. Wrapping seed potatoes in banana paper impregnated with abamectin or drenched with *Trichoderma asperellum* (Real Trichoderma®) led to suppression of soil *Meloidogyne incognita* densities by 87% and 68% in the field, and 86% and 40% in pots, respectively, which led to a 3.3- and 3.7-fold increase in yield in the field. This novel technology, also referred to as 'Wrap & Plant', presents a practical option for nematode management in potato under the resource-limited conditions of sub-Saharan Africa and offers potential for the targeted management of other soil-borne diseases.

Keywords – abamectin, biological control, environmental sustainability, lignocellulose matrix, *Meloidogyne* spp., plant-parasitic nematodes, *Pratylenchus* spp., soil disease management, sub-Saharan Africa, *Trichoderma asperellum*, Wrap & Plant.

Potato (*Solanum tuberosum*) is among the most important staple food crops globally. In sub-Saharan Africa, the crop is valuable for food security and income generation, and in Kenya it stands as the second most important crop after maize, where it is produced primarily by smallholder farmers (McEwan *et al*., 2021). In Kenya, the area

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Published with license by Koninklijke Brill BV | DOI: 10.1163/15685411-bja10367
 | ISSN: 1388-5545 (print) 1568-5411 (online)

cropped to ware potato is over 209 000 ha with yields of 8.3 t ha^{-1} (FAOSTAT, 2022), a figure way below its potential of >40 t ha^{-1} (NPCK, 2021). As a source of income, potato also contributes over US$ 0.5 billion to the Kenyan economy annually (CIP, 2019a), employing around 2.5 million people across the value chain (CIP, 2019b).

With its relatively short growing season, potato provides an ideal crop for food security regionally. Further, it can be planted year-round in some locations with 2-3 crops per annum, especially when planting early maturing cultivars, such as the regionally popular 'Shangi' (Kaguongo *et al*., 2014; Mburu *et al*., 2020). However, despite the regional importance of potato overall, production is declining, with Kenya alone witnessing a 40% decline within 10 years, even though the area cropped to potato increased 1.5-fold (FAOSTAT, 2022). This steady decline in productivity is due to several factors, such as pests and diseases (Riungu, 2011; Were *et al*., 2013), including potato cyst nematodes (PCN; *Globodera* spp.) and root-knot nematodes (RKN; *Meloidogyne* spp.) (Niere & Karuri, 2018). Although PCN were first reported in Kenya in 2015 (Mwangi *et al*., 2015) and have recently been shown to be highly damaging to Kenyan and regional potato production (Mburu *et al*., 2020), RKN are also a significant threat to production (Castagnone-Sereno, 2002; Khalil, 2013; Lima *et al*., 2018).

Root-knot nematodes are viewed as the most economically damaging group of plant-parasitic nematodes worldwide (Jones *et al*., 2013; Coyne *et al*., 2018a), and have been reported to cause losses of up to 90% of potato production and quality losses (Castagnone-Sereno, 2002; Khalil, 2013; Lima *et al*., 2018). The main species affecting potatoes in Africa and across the sub-tropics include *M. incognita*, *M. arenaria*, *M. javanica* and *M. hapla*, although there are an increasing number of records of other species, such as *M. chitwoodi* (Fourie *et al*., 1998; Coyne *et al*., 2018a). Root-knot nematodes are highly polyphagous with exceptionally broad host ranges, making it challenging to establish suitable and effective management options (Viaene *et al*., 2024). Previously, RKN management has relied heavily on synthetic chemical pesticides (Renčo & Kováčik, 2012). However, due to the withdrawal of the most effective of these pesticides for environmental and safety reasons (Onkendi *et al*., 2014), there is a great need for alternative management options for these pervasive pests. Techniques, such as host resistance, sanitation, soil tillage, cover crops, trap crops and green manures, among others, have been utilised in the management of RKN but effective management remains elusive, especially in the tropics and sub-tropics (Coyne *et al*., 2018a; Viaene *et al*., 2024). Consequently, interest in biologically-based options has grown considerably, with some products showing promising results, including the use of antagonistic fungi, such as *Trichoderma* species (Poveda *et al*., 2020; Temitope *et al*., 2020). In Kenya, *T. asperellum* strain TR900 has been produced and marketed (Real Trichoderma®) for effective use against RKN on a range of crops (RealIPM, 2021).

Abamectin is a nematicide with strong activity against a wide range of plant-parasitic nematodes and is marketed for use on a range of crops (Cao *et al*., 2016). Abamectin is derived from the natural fermentation of the soil bacterium *Streptomyces avermitilis* (Ōmura & Shiomi, 2007; Pitterna *et al*., 2009). It has relatively low toxicity to non-target beneficial arthropods, increasing its acceptance in relation to environmental safety (Lasota & Dybas, 1990; Khalil, 2013). However, the efficacy of abamectin is restricted by its insolubility in water and lipophilic nature, causing it to bind to organic matter leading to poor distribution and mobility in the soil (Cao *et al*., 2016). These characteristics can result in limited protection, as the compound may not reach the root zone, or when deployed as a seed coating the radicle may be rendered unprotected following germination (Cao *et al*., 2016; Khalil & Darwesh, 2019). Consequently, delivery of abamectin to the target root zone using an effective carrier would help to improve efficacy and prolong activity in the soil, extending its protection against plant-parasitic nematodes.

A field-deployable nutrient-rich biodegradable lignocellulose matrix, made from banana fibre, was identified and developed to deliver micro-dosages of nematicides to the target rhizosphere zone (Pirzada *et al*., 2020a, b; Ochola *et al*., 2022). The banana fibre paper has a high lignin content, which proved suitable for loading abamectin followed by a slow, sustained release over weeks. This technology, also known as 'Wrap & Plant', provides targeted delivery of high relative concentrations of nematicides to the rhizosphere, minimising environmental contamination and non-target effects (Ochola *et al*., 2022). It is being evaluated for use mainly on clonally propagated crops, with preliminary results on potato against PCN in Kenya showing great promise for nematode management (Ochola *et al*., 2020, 2022), and similarly positive results against the yam nematode (*Scutellonema bradys*) on yam in West Africa (Affokpon *et al*., 2018; Pirzada *et al*., 2023). The current study was undertaken to investigate the efficacy of the banana fibre paper

against RKN on potatoes under field conditions and determine its potential as a carrier for products such as the chemical nematicide abamectin and the fungal antagonist *T. asperellum*.

Materials and methods

Study sites

Research activities were conducted both in the field and in pots. The field trials were conducted in Mwea, Kirinyaga County, located in the foothills of Mount Kenya, approximately 90 km from Nairobi. Mwea is characterised by mean annual temperatures of 20.9-21.2°C and a mean annual rainfall of 900-1200 mm, with 285-300 rain days per year (Jaetzold *et al*., 2011). Mwea soils are predominantly clay-rich black cotton soils that are poorly drained and become easily waterlogged during heavy rains. The field trials were conducted under rainfed conditions at two locations, each over two consecutive growing seasons (April-July and September-December 2017, harvesting 110 days after planting); Field Trial 1 (0.58638°S, 37.37194°E; 1249 m above sea level (m a.s.l.)) and Field Trial 2 (0.37220°S, 37.15190°E; 1276 m a.s.l.). A pot trial was conducted in the screenhouse at Kenyatta University farm, Nairobi (1.14618°S, 36.96649°E; 1500 m a.s.l.), with a mean annual temperature of 26°C (range between 7 and 34°C) (Kenyatta University, 2017), between October and December 2017. The two field trial sites were selected further to establishing the presence of RKN from soil samples at levels of >50 infective second-stage juveniles (J2) per 200 ml soil (see below).

Treatments

The study included six treatments: *i*) banana paper impregnated with 100 ng abamectin sheet^{-1} (pre-treated); *ii*) banana paper drenched with commercial *T. asperellum* (Real Trichoderma®, RealIPM); *iii*) untreated banana paper; *iv*) soil drenched with Tervigo® (Syngenta, Nairobi, Kenya) (abamectin alone); *v*) soil drenched with commercial *T. asperellum*; and *vi*) absolute control (farmer practice). Certified potato seed tubers ('Shangi'), ranging from 35 to 45 mm, were sourced from the International Potato Center (CIP), Nairobi, Kenya. The banana paper sheets (L × W = 10.0 × 12.5 cm; density = 0.2757 g cm^{-3}) (Pirzada *et al*., 2020a, b; Ochola *et al*., 2022), were supplied by North Carolina State University, Raleigh, NC, USA. For the '*T. asperellum*-drenched banana paper' treatment, potato tubers were wrapped in non-treated banana paper, individually placed in the planting hole and each wrapped tuber was drenched with 300 ml of diluted *T. asperellum* suspension (1.5 × 10^4 cfu ml^{-1}). Similarly, 300 ml of diluted *T. asperellum* suspension was used to drench each planting hole where unwrapped potato tubers were planted; this formed the '*T. asperellum* alone' treatment. These two treatments were used to deliver the recommended application rate of 200 ml ha^{-1} of Real Trichoderma® suspension (1.0 × 10^9 cfu ml^{-1}). Soil drench with abamectin was achieved by drenching each potato seed tuber in the planting hole with 300 ml of diluted Tervigo® suspension (12 μg abamectin ml^{-1}) to deliver the recommended application rate of 160 g abamectin ha^{-1}. Drenching was achieved by applying half the treatment into the planting hole, and the remainder onto the surface after covering the seed with soil to ensure a homogeneous distribution. For the abamectin-treated and non-treated banana paper treatments, potato seed tubers were individually wrapped in the respective paper types and directly planted in the holes without any additional treatments. The absolute control reflected regular farmer planting conditions, without paper or chemical nematicide or fungal antagonist.

Field trial establishment

In the field trials, treatments were arranged in a complete randomised block design with four replications (plots) per treatment. Plots were prepared by hand-hoe and measured 3.75 × 3.90 m with a 1 m buffer between blocks and between plots within each block, respectively. Each plot contained 65 plants, spaced at 30 cm within each row, with rows 75 cm apart, forming a plant population of 44 444 plants ha^{-1}. A pre-plant application of diammonium phosphate (DAP) fertiliser was thoroughly mixed into the soil at a rate of 800 g plot^{-1} (500 kg ha^{-1}). To establish the initial nematode density (P_i) at each field site, a composite soil sample of *ca* 1 kg was collected from each plot using a hand trowel. The composite soil sample was collected from five random points per plot at a depth of 20-30 cm (Coyne *et al*., 2018b). Soil samples were labelled carefully, stored in a cool box and transferred directly to the *Nem*Africa laboratory at *icipe*, Nairobi, Kenya for processing. Samples were thoroughly mixed and sieved through a 1 mm mesh sieve before removing a 200 ml soil sub-sample for nematode extraction over 48 h using a modified Baermann tray method (Coyne *et al*., 2018b). Nematode suspensions were concentrated to 10 ml in plastic beakers using a 25 μm sieve,

identified to genus level for plant-parasitic nematodes and counted from a 2 ml aliquot in a counting dish under a dissecting microscope (Olympus-CX 22) at 40× magnification. Furthermore, tomato seedlings ('Moneymaker') were planted in 2 l plastic pots filled with nematode-infested non-sterile soil sampled from the field sites. Upon infection of the tomato plants and RKN development in the tomato roots, mature RKN females were gently isolated from the galled tomato roots and RKN were identified to species level based on Nad5 mtDNA (Janssen *et al*., 2016). Soil samples of 200 g plot^{-1} were sent to Kenya Agricultural and Livestock Research Organization, Nairobi, Kenya to determine basic soil characteristics. The soil characteristics of Field Trial 1 included soil pH (5.99-moderate acidic), total nitrogen (0.15%), total organic carbon (1.42%, moderate), and adequate nutrient levels. The soil characteristics of Field Trial 2 included soil pH (5.93, moderate acidic), total nitrogen (0.20%), total organic carbon (1.90%), and adequate nutrient levels.

Pot trial establishment

The pot trial included ten replicate plants ('Shangi') per treatment, with a single seed tuber planted per 5 l capacity pot, filled with autoclaved soil (from Field Trial 1 site) and sand in a 1:1 ratio. The six treatments, as used in the field trials, were arranged in a completely randomised design on the floor of the screenhouse. All treatments were administered in the same manner and application rates as for the field trials. One day before treatment, all pots were moistened to carrying capacity. Diammonium phosphate was applied at planting with 11.25 g pot^{-1} (500 kg ha^{-1}) by mixing into the soil prior to planting. The RKN inoculum was sourced from infested tomato roots obtained from the field. For the inoculum, galled tomato roots were uprooted from an infested crop in the field. Roots were transported to the laboratory and were gently washed free of soil. The roots were surface-sterilised by dipping them into 1.5% sodium hypochlorite (NaOCl) for a few seconds to ensure that no other nematodes were on the surface of the roots. The roots were rinsed under tap water, a few RKN females were randomly and gently extracted from the galled roots and used for RKN identification based on Nad5 mtDNA (Janssen *et al*., 2016). The roots were chopped into *ca* 0.5 cm pieces and macerated using a food blender at 1000 rpm for 5 s. The macerated roots were incubated for 1 week in the laboratory at room temperature, using modified Baermann trays to collect the J2. The freshly hatched J2 obtained from the RKN cultures were pooled and recovered daily into fresh tap water for 2-3 days until enough inoculum was available for all 60 pots. The inoculum was counted under the dissection microscope, pooled and adjusted to 250 J2 (20 ml)$^{-1}$. Each pot was inoculated at planting with 250 freshly hatched RKN J2 by pipetting 20 ml aqueous suspension into two small holes of 0.5 cm diam. and 5 cm deep. Following planting, pots received no irrigation for 2 days to enable treatments and nematodes to settle, and after that were irrigated daily with 500 ml pot^{-1} until harvest. The potatoes were harvested 110 days after planting. The trial was repeated once in time.

Plant growth and nematode damage parameters

At harvest, the mean number of stems, tuber number per plant, root weight (g) and tuber weight per plant were recorded from 14 randomly selected plants per plot in the field and from all pot plants, while tuber yield per hectare (t ha^{-1}) was estimated from the total tuber weight per plot in the field. Nematode soil densities were assessed at harvest (P_f) from a 200 ml sub-sample of soil samples obtained from each plot and for each pot, as described above for P_i, using the modified Baermann technique. Nematodes were observed under the compound microscope, counted as above and identified to genus for all plant-parasitic nematodes, except for RKN, whose species identity had been confirmed using Nad5 mtDNA. Nematode reproduction factor (RF) was calculated for the field and pot trials by dividing P_f by P_i.

Data analysis

All data were analysed using R (Version 4.2.3) statistical software (R Core Team, 2023).

For the field trials, data for *Tylenchus* (P_i and P_f), *Pratylenchus* (P_i and RF), free-living nematodes (P_i, P_f and RF), tuber weight, tuber number and stem number were subjected to a two-way analysis of variance (ANOVA) to investigate the main and interaction effects of season and treatment on each parameter. Prior to analysis, tuber weight data were square-transformed to conform to the requirements for normality (Shapiro-Wilk test: $P > 0.05$) and homogeneity of variances (Levene's test: $P > 0.05$); data for other parameters were not transformed.

Due to the binary nature of the proportion of sprouted potato tubers (sprouted *vs* non-sprouted), the data were fitted to a generalised linear model (GLM) with binomial

distribution to check for the main and interaction effects of season and treatment. Data for all the other nematode damage and plant growth parameters for both the field and pot trials were fitted to a GLM with Gaussian distribution to check for the main and interaction effects of season and treatment on each parameter in the field trials, or the main effect of treatment in the pot trial. When significant effects were detected at $P < 0.05$, group means were separated using Tukey's Honest Significant Difference (Tukey-HSD) test.

Results

Effect of treatments on potato tuber weight and yield

For the field trial, the number of tubers per plant in season 1 (6.1 tubers) was not significantly different from season 2 (6.2 tubers) ($F = 0.60$, $P = 0.44$); however, tuber number was significantly influenced by treatment ($F = 16.8$, $P < 0.001$), but with no interaction of season and treatment ($F = 1.1$, $P = 0.37$). Consequently, data were pooled across seasons prior to further analysis. More tubers per plant were produced in the *T. asperellum*, *Trichoderma*-paper- and abamectin-paper-treated plants compared to the non-treated absolute control (Fig. 1A). Potato tuber weight in season 1 of the field trial (478 g plant^{-1}) did not differ from season 2 (473 g plant^{-1}) ($F = 0.21$, $P = 0.65$); however, tuber weight was significantly influenced by treatment ($F = 26.6$, $P < 0.001$), but with no interaction of season and treatment ($F = 2.1$, $P = 0.09$). Thus, data were pooled across seasons prior to further analysis. Overall, while application of treatment significantly improved tuber weight ($\geqslant$437 g plant^{-1}) compared to non-treated absolute control (255 g plant^{-1}), *T. asperellum*, *Trichoderma*-paper and abamectin-paper treatments registered the highest tuber weight per plant (Fig. 1B).

Similarly to the field trial, more tubers per plant were recorded in the *T. asperellum*, *Trichoderma*-paper and abamectin-paper treatments ($\geqslant$4.9 tubers) compared to non-treated absolute control (2 tubers) in the pot trial ($\chi^2 = 46.2$, $P < 0.001$) (Fig. 1E). Furthermore, apart from the abamectin treatment, application of treatments to plants significantly improved potato tuber weight ($\chi^2 = 252.7$, $P < 0.001$) in the pot trial compared to the non-treated absolute control (Fig. 1F).

While the overall crop yield in season 2 of the field trial (16.0 t ha^{-1}) was significantly higher than season 1 (14.8 t ha^{-1}) ($\chi^2 = 12.9$, $P < 0.001$), and yield was significantly influenced by treatment ($\chi^2 = 878.4$, $P < 0.001$), there was no interaction between season and treatment ($\chi^2 = 8.67$, $P = 0.12$); thus, yield data were pooled across seasons prior to further analysis. Application of treatment boosted the crop yield by $\geqslant$140%, with abamectin-paper treatment recording the highest yield improvement (278%), relative to the non-treated absolute control (Fig. 2).

Effect of treatments on potato growth

The proportion of sprouted tubers in season 2 of the field trial (85.6%) was significantly higher than season 1 (71.3%) ($\chi^2 = 20.4$, $P < 0.001$); however, tuber sprouting was neither influenced by treatment nor was there a significant interaction between season and treatment ($\chi^2 \leqslant 5.9$, $P \geqslant 0.31$). A 100% tuber sprouting was recorded across treatments in the pot trial. The number of stems per plant did not vary between season 1 (6.4) and season 2 (6.3) of the field trial ($F = 0.37$, $P = 0.55$); however, stem number was significantly influenced by treatment application ($\chi^2 = 5042$, $P = 0.001$), but there was no interaction of season and treatment ($\chi^2 = 1.5$, $P = 0.20$). Overall, the *T. asperellum* and *Trichoderma*-paper treatments produced more stems per plant ($\geqslant$6.6) compared to the non-treated absolute control (5.7) (Fig. 1C). However, in the pot trial, the abamectin-paper treatment had a significantly higher stem number per plant compared to all other treatments ($\chi^2 = 4.1$, $P < 0.001$), while the absolute control had the least number (Fig. 1G).

Overall, application of the treatments significantly boosted plant root growth in both the field ($\chi^2 = 41.3$, $P < 0.001$) and pot ($\chi^2 = 67.9$, $P < 0.001$) trials, with the abamectin-impregnated paper treatment producing the highest root weight/plant in the field (6.0 g plant^{-1}) and pot (5.2 g plant^{-1}) trials, while the non-treated absolute control produced the least root weight in the field (2.9 g plant^{-1}) and pot (1.5 g plant^{-1}) trials, respectively (Fig. 1D and H).

Nematode densities and damage assessment

Results from the amplification of the Nad5 mtDNA region revealed that *Meloidogyne incognita* was the RKN species present in both the field and pot trials. In season 1 of the field trial, an overall P_i density of 402 nematodes (200 ml soil)$^{-1}$ was recorded prior to trial establishment; this was composed of RKN (13%), *Pratylenchus* spp.

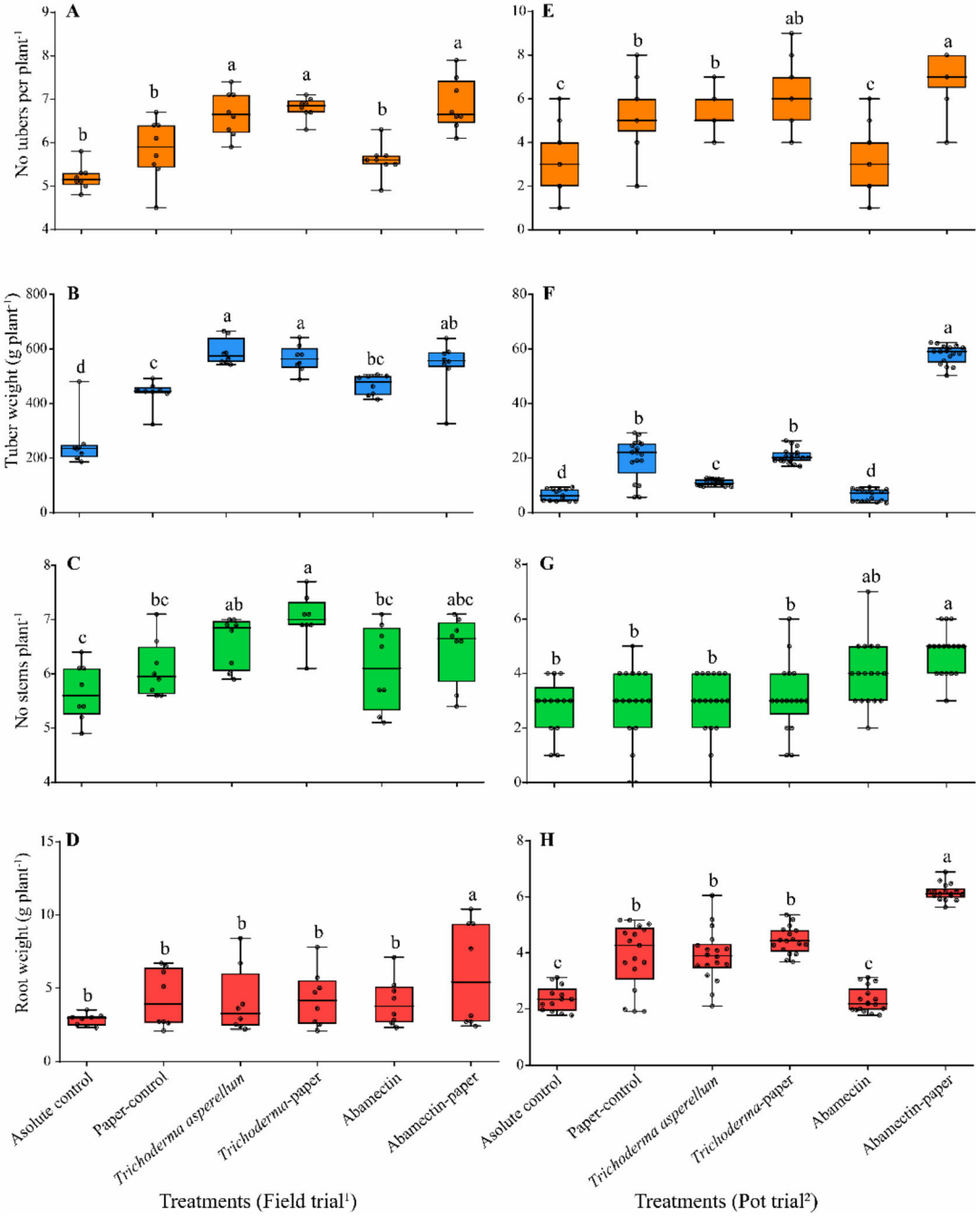

Fig. 1. Boxplots illustrating effect of treatments on growth of potato ('Shangi') in the field (left) and pot (right) trials. Within each graph, boxplots followed by same letter indicate no significant difference. Means separated by Tukey's [honestly significant difference (HSD)] test at $P < 0.05$. The bar in each box indicates the median, while bars at the extremes of the boxes indicate the 25th-75th percentiles. The ends of box whiskers indicate the minimum and maximum of the data points. Dots in each box show the data points from each replicate. [1] Season 1: April-July, 2017; Season 2: September-December, 2017; n = 2 trials × 4 replicate plots per treatment; 14 plants randomly selected per plot. [2] Pots were inoculated with 250 *Meloidogyne incognita* infective second-stage juveniles; n = 2 trials × 10 pots per treatment.

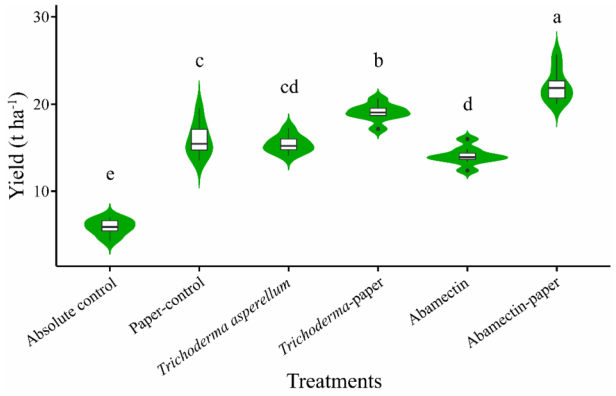

Fig. 2. Violin plots showing effect of treatment on yield of potato ('Shangi') under field conditions. Plots followed by the same letter indicate no significant difference. Means separated by Tukey's honestly significant difference (HSD) test at $P < 0.05$. Data from two seasons pooled prior to analysis; Season 1: April-July, 2017; Season 2: September-December, 2017; n = 2 trials × 4 replicate plots per treatment; 65 plants per plot.

(38%), *Tylenchus* spp. (34%) and other plant-parasitic nematodes (15%). A P_i density of 993 was recorded for season 2, which was composed of RKN (65%), *Pratylenchus* ssp. (15%), *Tylenchus* spp. (13%) and other plant-parasitic nematodes (7%). Overall, the RKN soil P_f density in season 1 (23.3) was significantly lower than season 2 (317) ($\chi^2 = 599.6$, $P < 0.001$). Similarly, there was an overall significant effect of treatment ($\chi^2 = 466$, $P < 0.001$) and an interaction of season and treatment ($\chi^2 = 392.6$, $P < 0.001$) on RKN P_f. Consequently, data on RKN soil P_f densities were analysed independently for each season. Root-knot nematode P_f densities were not influenced by treatment in season 1 ($\chi^2 = 7.9$, $P = 0.16$). However, in season 2, RKN P_f densities were significantly lower ($\chi^2 = 488$, $P < 0.001$) in all treatments compared to the non-treated absolute control (Table 1). While RKN reproduction was suppressed in both cropping seasons, the RKN RF in season 1 (0.39) was significantly lower than in season 2 (0.58) ($\chi^2 = 19.8$, $P < 0.001$). Similarly, there was an overall significant effect of treatment ($\chi^2 = 404.5$, $P < 0.001$), and a significant interaction of season and treatment ($\chi^2 = 55.2$, $P < 0.001$) on RKN RF. Application of the treatments in season 1 ($\chi^2 = 56.1$, $P < 0.001$) and season 2 ($\chi^2 = 1040.3$, $P < 0.001$) significantly suppressed RKN reproduction relative to control, respectively (Table 1).

While the soil P_f densities of *Pratylenchus* spp. ($\chi^2 = 7.0$, $P = 0.22$) and *Tylenchus* spp. ($F = 2.5$, $P = 0.07$) were not influenced by treatment application in season 1, the overall soil P_f densities of plant-parasitic nematodes were significantly supressed by treatment ($\chi^2 = 15.0$, $P = 0.01$) compared to control (Table 2). Furthermore, application of the treatment significantly supressed reproduction of *Pratylenchus* spp. ($F = 22.5$, $P < 0.001$), *Tylenchus* spp. ($\chi^2 = 33.0$, $P < 0.001$) and the total plant-parasitic nematodes ($\chi^2 = 485.4$, $P < 0.001$) compared to control (Table 2). Soil P_f and RF densities of *Pratylenchus* spp. *Tylenchus* spp. and free-living nematodes were not influenced by treatment in season 2 of the field trial ($P > 0.05$).

While there was a significant effect of treatments on the soil RKN P_f densities and RF in the pot experiment ($\chi^2 = 54.4$, $P < 0.001$), there was neither a significant effect of experiment repeat ($\chi^2 \leqslant 0.32$, $P \geqslant 0.57$), nor interaction of treatment and experiment repeat on RKN P_f or RF ($\chi^2 \leqslant 4.67$, $P \geqslant 0.46$), respectively. Consequently, data were pooled across experiment repeats prior to further analysis. Application of the treatment significantly suppressed RKN P_f and RF compared to non-treated absolute control; the abamectin-paper treatment produced the lowest P_f and RF (Table 1).

Discussion

Impregnation of banana fibre paper with either abamectin or *T. asperellum* and then wrapping seed tubers as a mechanism for delivering either a synthetic or a biologically-based nematicide to the target root zone of potato plants proved effective. Impregnated banana fibre paper provided strong suppression of *M. incognita*, significantly reducing nematode multiplication and leading to improved potato yields compared with the farmer practice control treatment. This was consistently demonstrated across two field sites over two consecutive seasons in Kenya, with the field data strongly complimented by the pot trial results on the effect of banana fibre paper against *M. incognita* and yield improvement. Over the duration of the growing season the paper gradually degraded over time in the field but could still be detected at 16 weeks (Fig. 3), as similarly observed by Ochola *et al.* (2022). The rate of abamectin release is dependent on the paper's chemical compositions of lignin, hemicellulose and cellulose, and the corresponding distribution of each component (Cao *et al.*, 2016). The higher lignin content in the bulk of the banana paper enabled the slow and sustained release of loaded abamectin (Cao *et al.*, 2016; Ochola *et al.*, 2022). Abamectin has poor mobility in soil due to its hydrophobicity and because it has a strong affin-

Table 1. Root-knot nematode (*Meloidogyne incognita*) soil densities (nematodes (200 ml soil)$^{-1}$ in potato ('Shangi') field and pot trials.

Treatment	Field trial						Pot trial	
	P_i		P_f		RF		P_f	RF
	Season 1*	Season 2	Season 1*	Season 2	Season 1	Season 2		
Absolute control	45±10	460±50 b	45±10	855±55 a	1.00±0.00 a	1.89±0.10 a	127±22 a	12.7±2.2 a
Paper-control	55±22	555±70 ab	25±13	300±22 b	0.38±0.13 b	0.55±0.03 b	45±5 bc	4.5±0.5 bc
Trichoderma asperellum	65±33	745±51 ab	25±15	208±11 bcd	0.28±0.14 b	0.28±0.00 c	83±7 ab	8.3±0.7 ab
Trichoderma-paper	50±6	615±67 ab	20±0	160±22 cd	0.42±0.05 b	0.26±0.04 c	76±7 b	7.6±0.7 b
Abamectin	50±17	870±24 a	20±12	265±13 bc	0.25±0.14 b	0.30±0.01 c	73±11 b	7.3±1.1 b
Abamectin-paper	55±10	620±134 ab	5±5	115±17 d	0.06±0.06 b	0.20±0.02 c	17±5 c	1.7±0.5 c

Values represent means±standard error; Within each column, means followed by different letter(s) indicate significant difference between treatments; * no significant difference between treatments. Means were separated using Tukey's HSD test at $P < 0.05$. Season 1: April-July, 2017; Season 2: September-December, 2017; n = 2 trials × 4 replicate plots per treatment; 65 plants per plot; P_i = initial population density; P_f = final population density; RF = reproduction factor. Pots were inoculated with 250 *Meloidogyne incognita* infective second-stage juveniles; n = 2 trials × 10 pots per treatment.

ity to organic matter. Although it binds tightly to banana paper, it is slowly released over time from the lignocellulose matrix as the paper deteriorates and decomposes in the soil (Pirzada *et al*., 2020a, b; Ochola *et al*., 2022). While RKN can penetrate the banana paper quite readily (Pirzada *et al*., 2020a, b), when provided a choice in *in vitro* experiments RKN appear to avoid having to do so (Tharp *et al*., unpubl. obs.). Additionally, roots and shoots readily pass through the paper as manufacture was designed with this trait in mind (Pirzada *et al*., 2020b). It has been observed that banana paper alone may help in the management of nematodes by disrupting chemical communication between the nematode and the potato plant, reducing the chances of infection and establishment in potato plants (Ochola *et al*., 2022; Tharp *et al*., unpubl. obs.).

Although it is likely that banana paper does not act as a substantial barrier to the nematode infection, the observation that root exudates (attractants) are tightly bound by the matrix suggests a potential disruption in plant-nematode communication (Ochola *et al*., 2022). Previous work has further revealed that the paper has no allelopathic properties or direct toxic effects on nematodes (Tharp *et al*., unpubl. obs.), further supporting the interruption of host-nematode communication as the primary impact of untreated banana paper on plant-parasitic nematodes.

This is the first report for the use of banana fibre paper in the field against RKN, as well as some limited indications of its use against other important nematode pests, such as *Pratylenchus* spp. As previously observed in field trials on *Globodera rostochiensis* (Ochola *et al*., 2022), RKN densities were consistently reduced with Wrap & Plant treatments and crop yield was significantly improved compared to farmer's practice. Applying either abamectin or *Trichoderma* through the Wrap & Plant platform resulted in ⩾3.3-fold increase in potato yields in RKN-infested fields. A particularly appealing feature for using banana fibre paper is its ability to adsorb ultra-low dosages of chemical compounds, such as abamectin, enabling the slow release of much lower field application levels of nematicides than recommended application rates (Cao *et al*., 2016). This approach enables the delivery of a low, but effective, dosage of abamectin. For example, it is estimated that the field delivery of abamectin using banana fibre paper is approximately 1000 times lower than the recommended application rates of Tervigo® (Ochola *et al*., 2022). In the current trials, abamectin-paper, although applied at a rate 625-fold lower than the recommended application rate of Tervigo® (abamectin-alone), was not only similarly effective at reducing nematodes, but led to a better crop yield than Tervigo®. This provides for a much more cost effective, as well as an environmentally sensitive, management option for RKN than the conventional application of synthetic pesticides.

More significantly, however, are the data that provide strong positive indications on the potential use of banana fibre paper for delivering biologically-based products. The effective delivery of a biologically-based product (Real Trichoderma®) further extends the potential use

Table 2. Soil densities of selected nematode genera (nematodes (200 ml soil)$^{-1}$) in potato ('Shangi') field trial (season 1).

Treatment	Free-living			*Pratylenchus*			*Tylenchus*			Total plant-parasitic nematodes		
	P_i*	P_f	RF	P_i*	P_f*	RF	P_i*	P_f*	RF	P_i*	P_f	RF
Absolute control	165±43	155±39 a	0.95±0.03 a	105±24	100±22	0.96±0.04 a	70±21	65±21	0.94±0.06 a	240±54	230±48 a	0.97±0.02 a
Paper-control	215±15	115±15 ab	0.53±0.03 b	165±59	95±42	0.54±0.05 b	125±26	65±15	0.52±0.04 b	385±78	200±47 ab	0.51±0.02 b
Trichoderma asperellum	178±10	70±13 ab	0.39±0.05 b	160±27	75±13	0.47±0.02 b	135±25	65±13	0.48±0.02 b	400±49	175±17 ab	0.44±0.02 bc
Trichoderma-paper	255±36	115±15 ab	0.46±0.02 b	155±35	65±19	0.41±0.04 b	225±26	100±22	0.43±0.04 b	540±88	225±43 a	0.41±0.03 bc
Abamectin	235±25	130±24 a	0.55±0.05 b	120±39	55±22	0.35±0.12 bc	85±38	35±15	0.45±0.21 b	315±105	130±52 ab	0.37±0.05 c
Abamectin-paper	180±22	25±5 b	0.14±0.01 c	215±15	25±5	0.12±0.02 c	180±67	30±6	0.20±0.05 b	530±88	65±13 b	0.12±0.01 d

Values represent means±standard error; Values with different letter(s) in a column indicate significant differences between treatments; * no significant difference between treatments. Means were separated using Tukey's HSD method at $P < 0.05$. Season 1 = April-July, 2017; P_i = initial population density; P_f = final population density; RF = reproduction factor; Total plant-parasitic nematodes = the sum of *Meloidogyne incognita*, *Pratylenchus* spp., *Tylenchus* spp. and other plant-parasitic nematodes.

of banana fibre paper, creating an even more environmentally sustainable alternative to synthetic pesticides. The demonstrated impact of *Trichoderma*-paper on potato yields and reduction of RKN in the field and pots provides compelling indications for the potential to adapt banana fibre paper for the delivery of a range of pest management products. Using the Wrap & Plant platform as a delivery vehicle, the biological control agent (*T. asperellum*) consistently outperformed the chemical nematicide (abamectin), strongly suggesting a new approach to delivery of biological moieties for reliable and successful nematode management and promotion of overall soil health. Therefore, determining why *Trichoderma* works so well in combination with the banana paper is a key question and intriguing, but one that needs to be assessed in future studies.

Here we demonstrate the effective application of chemical and biological nematicides on potato in Kenya, using banana fibre paper as a biodegradable carrier to manage RKN. Impregnating the banana fibre paper with abamectin appears to increase its efficacy, which is otherwise restricted by its insolubility in water and lipophilic nature. The current study further supports earlier studies that assessed the efficacy of abamectin-impregnated banana fibre paper against *G. rostochiensis* on potato (Ochola *et al*., 2022). The alternative use for a biological product further extends the potential of banana fibre paper as a vehicle for delivering crop protection products to the target root zone. We used banana paper sheets to wrap seed tubers and establish proof of concept for Wrap & Plant in the field, but this is not an optimal application method for ease of planting and utility. Based on our experience and farmers' recommendations, we are developing a pouch system for the Wrap & Plant platform that is more user-friendly and eases deployment in the field. The advent of the pouch system will reduce labour inputs, while the reduced application dosage of abamectin, compared to commercial applications, will lower the chemical input costs. The current study demonstrates that this novel technique provides for an effective and environmentally sensitive mechanism to address sustainable and affordable management of RKN on East African potato crops. However, it is too early yet to estimate costs as the technology is still in the research phase. However, cost is not prohibitive, or we would not be pursuing it. Assessing the impact of the banana fibre paper on soil microbiota, and the potential to control other pests and pathogens, will help decipher the full benefits of this promising regenerative innovation.

Fig. 3. An image illustrating the state of the banana fibre paper (arrow) at harvest.

Acknowledgements

This work was funded by donor contributions to the CGIAR Fund (https://www.cgiar.org/funders/) and, in particular, to the CGIAR Research Program for Roots, Tubers and Bananas (CRP-RTB). The authors thank the Bill & Melinda Gates Foundation (Grant nos OPP11188 10, OPP1196989; http://www.gatesfoundation.org) for their financial support. Special thanks are extended to *Nem*Africa staff, particularly Charei Munene (CM) for his input in the data analysis process. Real IPM, Kenya and Syngenta are gratefully acknowledged for their supply of Real Trichoderma® and Tervigo®, respectively, for use in the study.

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