RESEARCH REPORT

ASSESSING THE SUITABILITY OF GROWTH MEDIA IN THE GLASSHOUSE TO STANDARDIZE NEMATODE RESISTANCE SCREENING IN BANANA (*MUSA* SPP.)

Happyness J. Mpanda[1,2]*, Rony Swennen[3,4], Gloria Valentine Nakato[2,3], James Kisaakye[5], Kanan Saikai[5,6], Pavithravani B. Venkataramana[1], Allan Brown[2], and Danny Coyne[5]

[1]Department of Sustainable Agriculture and Biodiversity Conservation Management (SABEM), School of Life Science and Bioengineering (LiSBE), Nelson Mandela African Institution of Science and Technology (NM-AIST), P.O. Box 447, Arusha, Tanzania; [2]International Institute of Tropical Agriculture (IITA) c/o Nelson Mandela African Institution of Science and Technology (NM-AIST), P.O. Box 447, Arusha, Tanzania; [3]International Institute of Tropical Agriculture (IITA), Namulonge-Sendusu, 9 km Gayaza Zirobwe Road, P.O. Box 7878, Kampala, Uganda; [4]Department of Biosystems, KU Leuven, Belgium; [5]International Institute of Tropical Agriculture (IITA), *icipe* campus, P.O. Box 30772-00100, Nairobi, Kenya; [6]Ceradis B.V., Wageningen, Netherlands; *Corresponding author: mpandah@nm-aist.ac.tz

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ABSTRACT

Mpanda, H. J., R. Swennen, G. V. Nakato, J. Kisaakye, K. Saikai, P. B. Venkataramana, A. Brown, and D. Coyne. 2025. Assessing the suitability of growth media in the glasshouse to standardize nematode resistance screening in banana (*Musa* spp.). Nematropica 55: 65-72.

The burrowing nematode, *Radopholus similis*, is considered the most important nematode pest threatening banana production worldwide. Among the various nematode management strategies available, host resistance is one of the most promising. However, there is little information on host resistance in bananas against nematodes, including *R. similis*, which is partly explained by the inconsistency of the results and the resistance status of cultivars.

To reduce such inconsistency, it would be beneficial to use a standardized detection protocol, utilizing appropriate planting substrates that allow the penetration and reproduction of nematodes without interfering with plant growth. Sterilized forest soil has commonly been used, but it limits the penetration of nematodes into the host roots due to its fine texture. Consequently, a more suitable substrate could improve the efficiency of screening for host resistance of bananas against nematodes. In this study, *R. similis* was used as a model nematode to evaluate cocopeat and river sand as alternative substrates to forest soil that is normally used in pot evaluations. In the pots, *in vitro* plants of the cultivars Yangambi Km5 and Grande Naine were planted, representing resistant and susceptible genotypes, respectively. The findings revealed that different substrates showed a differential impact on the growth parameters of the plants (underground biomass), allowing the penetration and reproduction of *R. similis* within the roots of banana plants. A high growth of plants in sterilized forest soil was observed, followed by cocopeat, and then river sand. In contrast, plants grown in river sand showed a higher number of *R. similis* in the roots and a greater reproduction rate, compared to those grown in cocopeat and sterilized forest soil. Although cocopeat favored plant growth, it hindered the penetration of *R. similis* and reduced reproduction rates. Moreover, there was a high proportion of root loss in the harvest due to the strong adherence of cocopeat to the roots. Therefore, it is suggested to use river sand as the most appropriate substrate to evaluate the response of banana genotypes to *R. similis* infection under greenhouse conditions. This would improve the efficiency of resistance evaluations of bananas against nematodes.

Key words: Burrowing nematode, conventional breeding, growth media, host plant resistance, nematode management

RESUMEN

Mpanda, H. J., R. Swennen, G. V. Nakato, J. Kisaakye, K. Saikai, P. B. Venkataramana, A. Brown, y D. Coyne. 2025. Evaluación de la idoneidad de medios de cultivo en invernadero para estandarizar la evaluación de resistencia a nematodos en banano (*Musa* spp.). Nematropica 55: 65-72.

El nematodo barrenador, *Radopholus similis*, se considera la plaga de nematodos más importante que amenaza la producción de banano a nivel mundial. Entre las diversas estrategias de manejo de nematodos disponibles, la resistencia del huésped es una de las más prometedoras. Sin embargo, hay poca información sobre la resistencia del hospedero en banano contra *R. similis*, lo que se explica en parte, por la inconsistencia de los resultados y el estado de resistencia de los cultivares. Para reducir tal inconsistencia, sería beneficioso el uso de un protocolo de detección estandarizado, utilizando sustratos de siembra apropiados, que permitan la penetración y reproducción de nematodos sin interferir con el crecimiento de las plantas. Comúnmente se ha utilizado suelo forestal esterilizado, pero limita la penetración de los nematodos en las raíces del huésped debido a su textura fina. En consecuencia, un sustrato más apropiado podría mejorar la eficiencia del cribado de resistencia del huésped del banano contra *R. similis*. En este estudio, se evaluó la turba de coco y la arena de río como sustratos alternativos al suelo forestal que normalmente se utiliza en evaluaciones en macetas. En las macetas se sembraron plantas in vitro de los cultivares Yangambi Km5 y Gran Enano, como genotipos resistentes y susceptibles, respectivamente. Los hallazgos revelaron que diferentes sustratos mostraron un impacto diferencial en los parámetros de crecimiento de las plantas (biomasa subterránea), permitiendo la penetración y reproducción de *R. similis* dentro de las raíces de las plantas de banano. Se observó un alto crecimiento de plantas sembradas en el suelo forestal esterilizado en autoclave, seguido de turba de coco y luego arena de río. En contraste, las plantas sembradas en arena de río mostraron mayor número de *R. similis* en las raíces y una mayor tasa de reproducción, en comparación con las sembradas en turba de coco y suelo forestal esterilizado. Aunque la turba de coco favoreció a el crecimiento de las plantas, impidió la penetración de *R. similis* y redujo las tasas de reproducción.

Además, hubo una alta proporción de pérdida de raíces en la cosecha debido a la fuerte adherencia de la turba de coco a las raíces. Por lo tanto, se sugiere usar la arena de río como el sustrato más apropiado para evaluar la respuesta de los genotipos de banano a la infección por *R. similis* en condiciones de invernadero. Esto mejoraría la eficiencia de las evaluaciones de resistencia del banano contra los nematodos.

Palabras clave: Manejo de nematodos, medios de crecimiento, mejoramiento convencional, nematodo barrenador, resistencia de la planta huésped

INTRODUCTION

Bananas and plantains (*Musa* spp.) are grown in tropical and subtropical regions with worldwide socio-economic importance. *Musa* spp. production contributes to a major portion of the diet and is a key source of income for smallholder farmers in the tropics and subtropics. In 2023, global banana production reached 195.73 million tons (MT), an increase from 191.37 MT in 2022 (FAOSTAT, 2023). Globally, Uganda is the second-largest and Tanzania is the ninth-largest banana-producing country and are the first and second in the East Africa region.

Despite its importance, banana production is constrained by a range of pests and diseases, such as plant parasitic nematodes (*Radopholus similis*, *Meloidogyne* spp., *Pratylenchus* spp.), weevils (*Cosmopolites sordidus*), Fusarium wilt (*Fusarium oxysporum* f. sp. *cubense*), Xanthomonas wilt (*Xanthomonas vasicola* pv. *musacearum*), black Sigatoka (*Pseudocercospora fijiensis*) (Viljoen et al., 2017) and banana bunchy top disease (BBTD)

(Shimwela et al., 2022). Nematodes are significant biotic constraints affecting bananas and are a major factor contributing to production decline (Sikora et al., 2018). Globally, 30-60% loss in banana yield is attributed to nematode infection and 50% in Africa (Coyne et al., 2018; Sikora et al., 2018). While multiple nematode species invariably affect bananas and mostly as a species complex, including *Pratylenchus goodeyi, P. coffeae*, *Helicotylenchus multicinctus*, and *Meloidogyne* spp., *R. similis* is generally regarded as the most destructive (Coyne, 2021; Sikora et al., 2018). Females and juveniles are the damaging stages of this migratory endo-parasite, which penetrates the plant roots, feeds, multiplies, and migrates within the banana root system, resulting in necrotic lesions, poor root development, and reduced nutrient uptake. The damage caused to the roots leads to reduced plant anchorage, and consequently toppling of bunch-bearing stems, especially during storms (Coyne et al., 2018; Sikora et al., 2018).

Several management strategies against nematode infection in bananas have been proposed, including use of nematicides, healthy planting materials, botanicals, biological control, and host plant resistance (Sikora et al*.,* 2018). While nematicides such as carbofuran and fenamiphos are effective for nematode control, they have health and environmental concerns (Gallegos-Avila et al*.,* 2010), and their use is discouraged or even banned (WHO, 2010). The use of nematode resistance in bananas has received varied attention, primarily for deployment against *R. similis*, and against other nematode pests, with varied success (Sikora et al., 2018). One difficulty, however, is the generation of hybrids combining host plant resistance with desired agronomic and quality traits. The development of such resistant cultivars has been achieved through conventional breeding with repeated artificial pollination. This resistance is primarily expressed as either lack of (or reduced) penetration of the plant root system by the nematodes with failure to develop and multiply. The identification of resistance sources in banana parental genotypes is a key initial step in conventional breeding. Different banana breeding programs have developed standardized screening protocols but with variable and conflicting results in host response. The single root inoculation method was developed at the International Institute of Tropical Agriculture (IITA) to improve screening efficiency (De Schutter et al., 2001), which was later modified to multiple root inoculation (Coyne & Tenkouano, 2005), while others have used pot methods (Moens et al., 2003; Plowright et al., 2013; Van den Bergh et al., 2002). Both approaches continue to show inconsistency and variability in host response. Thus, an effective protocol which supports nematode penetration and multiplication to enable consistent differentiation between resistant and susceptible genotypes is needed. In particular, this involves reduced variability associated with experimental error to obtain better evaluation of the genetic potential of the plant material. For this, the use of suitable potting media is critical for reliable and useful conclusions about the host plant. Forest soil has been commonly used for glasshouse evaluations but the challenge is its low efficiency in supporting nematode penetration into the roots of the host plant due to its fine texture. This results in a poor interaction between nematode and plant host (Shane & Barker, 1986), leading to unreliable conclusions on the host responses. Therefore, in this study, we focused on evaluating the suitability of alternative growth media, such as cocopeat and river sand to autoclaved forest soil in supporting *R. similis* penetration and reproduction in tissue-cultured banana plantlets under glasshouse conditions.

MATERIALS AND METHODS

Banana plants

Two banana genotypes were used in this study, the nematode-resistant cv. Yangambi Km5 and susceptible cv. Grande Naine. Banana plants, produced through the shoot-tip culture technique (Vuylsteke, 1998), were sourced from the tissue culture laboratory of IITA, Arusha, Tanzania. Plants at the deflasking stage were washed free of rooting media and planted in plastic seedling trays with 70 planting holes filled with cocopeat (sourced from Datieco Co., Arusha, Tanzania in the form of blocks). Cocopeat blocks were hydrated using water overnight and treated with calcium nitrate for seedling propagation use, steam sterilized for 2-3 hours and allowed to cool for a day before planting. Plants were maintained in the humidity chamber (RH > 90%, at 25 ± 2°C)

and received 30 ml NPK 19:19:19 per plant once a week for 4 weeks. The plants were removed from the humidity chamber and placed on the bench for one week to acclimatize in the glasshouse prior to use in the experiment.

Growth media

Forest soil was sourced from the Tanzania Agricultural Research Institute (TARI) - Tengeru, Arusha, Tanzania. The top forest soil was randomly collected and steam-sterilized in a metallic drum for 2-3 hours and set aside to cool for 5 days before use. The soil was sandy loam (57% sand, 35% silt, and 8% clay) with a pH of 6.08, organic carbon (OC) of 1.55%, Ca 15.9, Mg 5.4, K 2.6 meq/100 g, Fe 83, Cu 0.42, Zn 3.17, and Mn 211.95 mg/kg.

River sand was purchased from a local supplier at Makumira, Arusha, Tanzania. Prior to use, sand was sieved through an 840 µm test sieve, steam-sterilized for 2-3 hours and left to cool for 5 days. Compressed dried cocopeat plugs/pellets 3 cm in diameter and 1 cm deep were used, which comprised course <1 mm particle coir and once hydrated swelled in size but were maintained in shape by a biodegradable net. Cocopeat plugs used in this study were purchased from Ecofarm Africa Connections Ltd., Nairobi, Kenya. Prior to use in the experiment, the cocopeat plugs were placed in a plastic tray (42 × 30 × 5 cm: L × W × H) filled with sterile distilled water to hydrate the plugs.

Nematode inoculum and experimental design

The *R. similis* inoculum used was previously isolated from infected banana plants grown in the field and maintained as monoxenic cultures in the incubator on carrot discs (Coyne et al., 2014). After acclimatization, banana plants were removed from the seedling trays, carefully washed in tap water to remove all cocopeat media and immediately planted singly in 400 ml plastic pots (transparent disposable glass) containing 200 ml of either sterile forest soil or river sand. Before transplanting, three holes of ~0.3 cm were made at the bottom of the plastic pot to drain excess water during plant watering. Another set of plants was planted in the cocopeat plugs previously soaked in water. For each banana cultivar, a total of 24 plants were transplanted to each of the growth media. At 2 weeks post transplanting, half of the plants within each media type and for each banana cultivar (12 plants) were inoculated with 500 *R. similis* (females and infective juveniles), while the remaining 12 plants were each inoculated with 1,000 *R. similis,* according to Speijer & De Waele (1997). The experiment was conducted twice; plants were arranged in a randomized complete block design with six replications in the glasshouse (RH > 90%, at 25 ± 2°C) and received 50 ml of water daily. Once a week until experiment termination, each plant received 50 ml of water soluble (1.5 g/L) poly-feed starter NPK (19:19:19) fertilizer sourced from Balton-Arusha, Tanzania.

Experiment termination

Half the number of the plants from each treatment (6 plants) were terminated at 2 weeks post-nematode inoculation (PNI); these plants were used to assess nematode penetration into the banana roots. The remaining plants were terminated at 8 weeks PNI and were used to assess nematode multiplication and development.

To assess for nematode penetration, the plants were carefully removed from the growth media, the roots gently washed under running tap water to remove any adhering media and blotted with tissue paper to remove excess water. The roots were cut from the banana corm using a scalpel blade and placed into a 50 ml plastic beaker. Nematodes in the banana roots were stained with acid fuchsin (Byrd et al., 1983) and total number of nematodes per plant root system quantified under a stereo microscope (Motic X25 magnification).

To understand nematode multiplication and development, the plants were removed from growth media at 8 weeks PNI, washed under running tap water, and carefully cut from the corm as described above. The root, shoot, and pseudostem fresh biomass weight was measured using weigh balance. Roots were chopped into ~0.5 cm, thoroughly mixed, and nematodes extracted from a 5 g root sub-sample over 48 hours using a modified Baermann method (Coyne et al., 2018). After extraction, the nematode suspension was reduced to a uniform 25 ml for nematode quantification from 3 × 2 ml aliquots under the stereo microscope.

Data analysis

Plant growth parameters (belowground biomass), nematode penetration, and reproduction data were analyzed using GenStat Data 21st Edition Software (64-bit). A linear mixed model (REML) was used to test the factor effects, while Fisher's protected test ($p = 0.05$) was performed for means separations. Data from the two experiments were combined and analyzed together. None of the data was transformed prior to analysis.

RESULTS

Plant growth biomass

At 2 and 8 weeks PNI, belowground biomass (root and rhizome weight) was significantly different for media type, banana cultivar, and inoculum level ($p \leq 0.001$). Also, an interaction effect was observed on media type and banana cultivars; media type and nematode inoculum level; media type, banana cultivar, and inoculum level. Banana plants grown in pots with autoclaved forest soil had heavier belowground biomass, followed by cocopeat plugs, with the lowest in river sand. Furthermore, at 2 weeks PNI, plants had lower biomass at the 500 nematode inoculum level compared to those inoculated with 1,000 nematodes (Fig. 1A). In contrast, lower plant biomass was recorded with 1,000 nematode inoculum at 8 weeks PNI (Fig. 1B). The cv. Yangambi Km5 had higher belowground biomass compared to cv. Grande Naine, irrespective of nematode inoculum levels at 2 and 8 weeks PNI (Fig. 1A-B).

Nematode penetration and reproduction

Nematode penetration at 2 weeks PNI and reproduction at 8 weeks PNI differed among the media type, banana cultivar, and nematode inoculum level ($p \leq 0.001$). Also, the interaction effect was observed on media type and banana cultivars; media type and nematode inoculum level; media type, banana cultivar, and inoculum level. Generally, fewer nematodes penetrated and reproduced in banana roots grown on autoclaved forest soil than in cocopeat plugs, with the highest penetration and reproduction on river sand. Higher nematode counts were observed in cv. Grande Naine than in cv. Yangambi Km5. Furthermore, higher nematode penetration at 2 weeks PNI was observed on plants inoculated with 500 nematodes, while higher reproduction at 8 weeks PNI was on 1,000 nematode inoculum levels (Fig. 2A-B).

DISSUSSION

The current study observed higher nematode penetration and reproduction in cv. Grande Naine than in cv. Yangambi Km5, regardless of the growth media and the level of nematode inoculum applied, in agreement with other studies (Pinochet & Rowe, 1978; Wehunt et al., 1978). Furthermore, the heavier belowground biomass was recorded in cv. Yangambi Km5 than in cv. Grand Naine for both media and inoculum levels, which displayed less nematode infection compared to cv. Grand Naine.

This study highlighted river sand as an appropriate media for nematode screening, which allowed higher numbers of nematodes to penetrate and reproduce than in either cocopeat plugs or autoclaved forest soil, in agreement with other studies (Kim et al., 2017; Moore & Lawrence, 2013; Stolzy & Van Gundy, 1968). More nematodes penetrated banana roots when an inoculum of 500 nematodes was used compared with 1,000 nematodes, likely due to reduced competition among nematodes (Hashmi et al*.,* 1994; Pariyar et al*.,* 2010; Sayre, 1958). With the lower inoculum, there was more successful penetration into the banana roots with nematodes acquiring more nutrients to feed, reproduce, and multiply (Jiskani et al*.,* 2008). This resulted in plants inoculated with a lower number of nematodes exhibiting lower plant biomass than plants inoculated with a higher number of nematodes.

Belowground plant biomass was heavier on autoclaved forest soil compared to cocopeat plugs and river sand at 2 and 8 weeks PNI. The inverse relationship between nematode penetration and reproduction with belowground biomass was also shown. Nematode penetration and reproduction were low with autoclaved forest soil, reflecting the findings of Shane & Barker (1986). Although

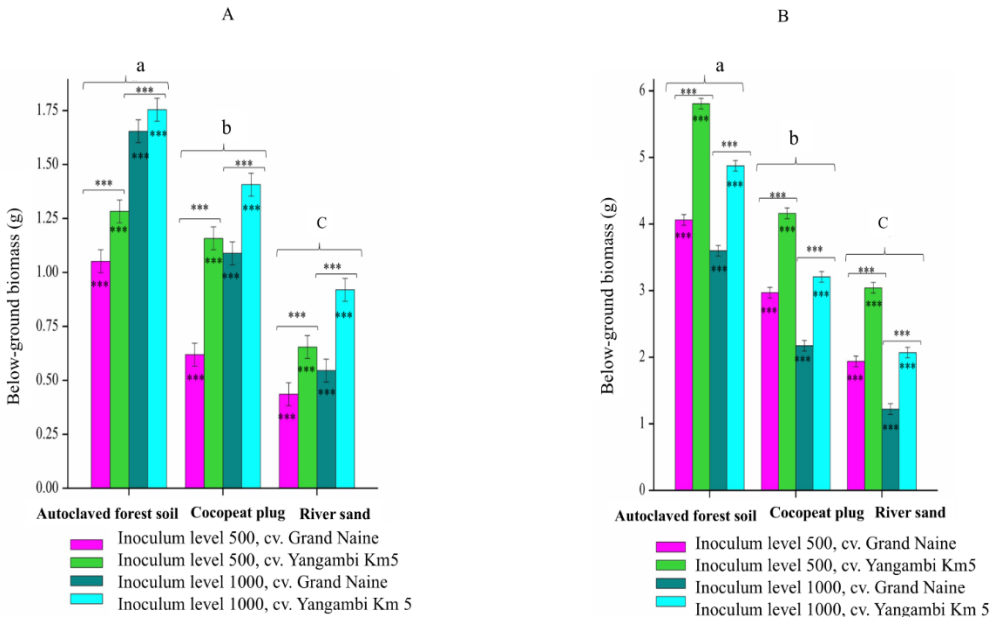

Figure 1. Belowground biomass response of two banana cultivars (Grande Naine and Yangambi Km5) grown on three potting media types (autoclaved forest soil, cocopeat plug, and river sand) at two *Radopholus similis* inoculum levels (500 and 1000), 2 weeks (A) and 8 weeks (B) post-nematode inoculation; different letters indicate significant differences based on Fisher's protected test at p = 0.05; *** significant at p ≤ 0.001.

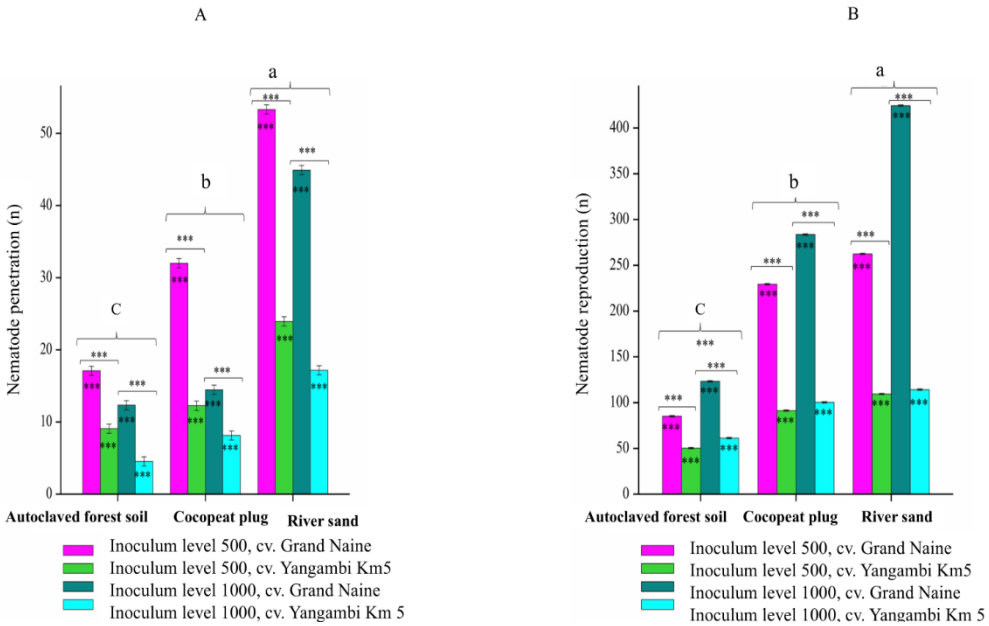

Figure 2. Nematode penetration and reproduction of two banana cultivars (Grande Naine and Yangambi Km5) grown on three media types (autoclaved forest soil, cocopeat plug and river sand) and with two *Radopholus similis* inoculum levels (500 and 1000) at two weeks (A) and 8 weeks (B) post nematode inoculation; different letters indicate significant differences based on Fisher's protected test at p = 0.05; *** significant at p ≤ 0.001, n = number of nematode penetrated and reproduced in the banana roots.

cocopeat enhanced plant growth due to its ability to allow root aeration and water retention, nematode root penetration and ultimate reproduction were less. The use of cocopeat also causes difficulties with root assessment, as the cocopeat fibers attach to the roots, which were difficult to separate from the roots, especially the finer roots of <1 mm in diameter. This led to the loss of many finer roots during washing and exclusion from the assessment. Cocopeat may, therefore, be a good substrate for seedling propagation but is not suitable for phenotyping for nematode resistance in bananas.

The current study showed that, irrespective of the nematode inoculum (500 or 1,000), river sand media led to higher nematode penetration and reproduction in banana roots, enabling greater differentiation of banana genotype response challenged for nematode resistance under glasshouse conditions. Consequently, this supports improved efficiency of banana breeding programs in developing and releasing of nematode-resistant cultivars, and as such is recommended for use when screening for nematode resistance.

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