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Research Article

DEVELOPMENT OF A RAPID BIOASSAY FOR THE EVALUATION OF THE NEMATICIDAL EFFICACY OF AQUEOUS PLANT EXTRACTS AGAINST PHYTOPARASITIC NEMATODES IN DALOA, COTE D'IVOIRE

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ABSTRACT

Phytoparasitic nematodes cause severe yield losses in vegetable crops across sub-Saharan Africa. Conventional bioassay methods for evaluating plant-based nematicides are time-consuming and resource-intensive, limiting their use in low-capacity research settings. This study developed a rapid, reproducible, low-cost laboratory bioassay and evaluated the nematicidal efficacy of aqueous leaf extracts from three plant species *Chromolaena odorata*, *Tithonia diversifolia*, and *Nicotiana tabacum* against naturally occurring phytoparasitic nematodes. Rhizospheric soil was collected from a severely infested pepper (*Capsicum annuum*) field (100% disease incidence; gall index 5) at the University Jean Lorougnon Guédé experimental site. Aqueous extracts (10 g dry powder L^{-1}) were applied individually and in combination to 100 g sieved soil aliquots in Petri dishes, alongside a distilled water negative control and an oxamyl positive control. Nematodes were extracted using a modified Baermann funnel technique after 48 h incubation at 28 ± 2°C and enumerated by Malassez chamber microscopy. Initial nematode density was approximately 400 nematodes cm^{-3}. All botanical treatments significantly reduced populations relative to the control (ANOVA, $p < 0.05$). Reduction rates reached 62.5% (*C. odorata*), 66.7% (*T. diversifolia*), 83.5% (*N. tabacum*), and 93.3% for the combined extract statistically equivalent to oxamyl (96.7%, $p > 0.05$). These results demonstrate that the three plant species possess significant nematicidal potential, and that their combination achieves near-equivalent efficacy to synthetic nematicides. The proposed bioassay constitutes an accessible and efficient screening tool for botanical nematicide candidates in tropical research contexts.

Keywords: Bionematicide, *Capsicum annuum*, Meloidogyne spp Test, Bioassay, ANOVA.

INTRODUCTION

Phytoparasitic nematodes represent one of the most economically significant constraints in global crop production, inflicting cumulative annual losses estimated at USD 80-118 billion worldwide (Elling, 2013; Jones *et al.,* 2013). In sub-Saharan Africa, root-knot nematodes (*Meloidogyne* spp.) are particularly damaging to vegetable crops, with reported yield losses of 20–50% in susceptible cultivars under severe infestation conditions (Onkendi *et al.,* 2014; Coyne *et al.,* 2018). In Côte d'Ivoire, pepper (*Capsicum annuum* L.) and tomato (*Solanum lycopersicum* L.) crops are among the most severely affected, with *Meloidogyne incognita* (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood being the dominant pathogenic species recorded in the Haut-Sassandra region (Koffi *et al*., 2017). The conventional management strategy for root-knot nematodes has historically relied on broad-spectrum synthetic nematicides such as oxamyl, carbofuran, fenamiphos, and 1,3-dichloropropene. While chemically effective, these compounds carry substantial drawbacks: high acute mammalian toxicity, environmental persistence, groundwater contamination risk, and accelerating resistance development in target nematode populations (Chitwood, 2002; Dita *et al.,* 2006). Furthermore, the progressive regulatory withdrawal of key synthetic nematicides in European and increasingly in African markets creates an urgent need for the identification of effective,

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environmentally compatible alternatives (Oka, 2010). Botanical nematicides have emerged as a promising component of integrated nematode management strategies. A substantial body of literature documents the nematicidal properties of plant secondary metabolites including alkaloids, terpenes, polyphenols, flavonoids, and glucosinolates against *Meloidogyne* spp. and other economically important nematode genera (Chitwood, 2002; Oka, 2010; Ntalli and Menkissoglu-Spiroudi, 2011). Several studies conducted in West Africa have specifically highlighted the efficacy of locally available plant species: Kassi *et al.* (2014) demonstrated the antifungal and biopesticidal activity of a commercial preparation (NECO) derived from *Ocimum gratissimum* L. against black leaf streak disease of plantain banana in Côte d'Ivoire, while Koffi *et al.* (2017) established that combined application of castor (*Ricinus communis* L.) and cattle manure significantly suppressed root-knot nematode populations on tomato, reducing gall indices by up to 65%.

Notwithstanding this accumulating evidence, the translation of laboratory-scale findings into practical nematode management recommendations remains hampered by the absence of standardized, rapid, and reproducible screening protocols adapted to the resource constraints of agricultural research institutions in developing countries. Conventional bioassay methods including pot experiments, greenhouse trials, and in vitro egg mass/larval mortality assays typically require several weeks to months, specialized equipment, and precisely quantified nematode inocula (Moens *et al.*, 2009). These requirements substantially limit the throughput of candidate plant screening programs. The present study addresses this methodological gap by proposing a rapid, Petri dish-based soil bioassay protocol that enables the preliminary evaluation of nematicidal plant extracts within a five-day experimental window. This approach exploits the active migration behavior of nematodes in soil as the endpoint measurement, thereby eliminating the need for axenic nematode cultures or controlled inoculation. Three plant species of demonstrated or hypothesized nematicidal relevance were selected as test candidates: *Chromolaena odorata* (L.) R.M. King & H. Rob., *Tithonia diversifolia* (Hemsl.) A. Gray, and *Nicotiana tabacum* L. The study (i) characterizes the nematode infestation context at the experimental site, (ii) quantitatively evaluates the nematicidal efficacy of individual and combined aqueous extracts against naturally occurring nematode populations, and (iii) benchmarks the biological activity of botanical treatments against the synthetic nematicide oxamyl (Vydate 3G).

MATERIALS AND METHODS

Study site and experimental crop characterization

The study was conducted at the experimental agricultural site of the University Jean LOROUGNON GUEDE (UJLoG), located in Daloa, Haut-Sassandra Region, Côte d'Ivoire (geographic coordinates: approx. 6°54'N, 6°27'W; altitude: 285 m a.s.l.; annual rainfall: 1,200–1,400 mm; mean annual temperature: 26.5°C). The experimental parcel was a 210 m² pepper (*Capsicum annuum* L.) field with no prior chemical nematicide application during the current cropping cycle. Assessment of nematode infestation status was performed prior to soil sampling. A zigzag sampling scheme was applied to 50 randomly selected pepper plants out of a total census of 710 individuals. Root gall severity was scored using the standardized 0–5 rating scale of Taylor & Sasser (1978), where: 0 = no galls, 1 = 1–2 galls, 2 = 3–10 galls, 3 = 11–30 galls, 4 = 31–100 galls, and 5 = more than 100 galls or near-complete root destruction. Disease incidence (DI) was calculated as:

$$DI\ (\%) = (\text{Number of galled plants} / \text{Total plants sampled}) \times 100$$

Soil sampling and preparation

Rhizospheric soil samples were collected from the 0–15 to 15–30 cm depth horizon around the root zone of 10 representative pepper plants selected along the zigzag transect. The samples were bulked into a single composite sample, homogenized, and passed sequentially through 2 mm and 90 µm mesh sieves to remove coarse roots, organic debris, and gravel. The sieved soil fraction was then divided into 100 g aliquots distributed into 90 mm diameter Petri dishes, which served as the experimental units for treatment application.

Plant material: collection, drying, and powder preparation

Three plant species were selected based on prior evidence of nematicidal or pesticidal activity in the scientific literature: (1) *Chromolaena odorata* (L.) R.M. King and H. Rob. (Asteraceae) invasive weed widely distributed across sub-Saharan Africa, known to contain pyrrolizidine alkaloids, terpenes, and phenolic compounds with documented antifeedant and pesticidal properties (Paduano *et al*., 2014); (2) *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae) Mexican sunflower, reported to contain sesquiterpene lactones (tagitinins, diversifolins) and diterpenes with demonstrated nematicidal and allelopathic activity (Adeyemi *et al.,* 2014; Sotelo *et al*., 2020); (3) *Nicotiana tabacum* L. (Solanaceae) tobacco, whose nematicidal properties are primarily attributed to nicotine, nornicotine, and anabasine alkaloids that disrupt nematode acetylcholinesterase and neuromuscular function (Chitwood, 2002; Hollingsworth *et al.*, 1994). *C. odorata* and *T. diversifolia* were harvested from fallow land within the Daloa commune; *N. tabacum* leaves were obtained

from the local market. Mature, disease-free leaves were selected, washed under running tap water, and shade-dried at ambient temperature (28 ± 2°C) for seven days to a moisture content below 12% (estimated by periodic weighing until mass stabilization). Dried leaves were ground to a fine powder using an electric mill and stored in sealed, airtight glass containers at room temperature in the dark until use.

Preparation of aqueous plant extracts and nematicide reference solution

Aqueous extracts were prepared by dissolving 10 g of each plant powder in 1 L of sterile distilled water, vigorously stirred for 5 minutes, and allowed to stand for 30 minutes at ambient temperature before application. This extraction ratio (10 g L^{-1} = 1% w/v) corresponds to concentrations routinely used in comparative botanical nematicide screening (Oka, 2010). No further filtration was applied to retain the full spectrum of water-soluble compounds. The combined extract (Tcombi) was prepared by mixing equal volumes (1:1:1, v/v/v) of the three individual stock extracts. The positive control nematicide solution was prepared by dissolving 10 g of commercial oxamyl (Vydate 3G, active ingredient: oxamyl 3%, DuPont) in 1 L of distilled water. Oxamyl is a carbamate nematicide that inhibits acetylcholinesterase activity and is registered for soil application against *Meloidogyne* spp. in vegetable crops (Chitwood, 2002). The negative control consisted of sterile distilled water only.

Experimental design and treatment application

A completely randomized design (CRD) was adopted with six treatments. Each treatment was replicated three times, yielding 18 Petri dish experimental units (Table 1). The six treatments were:

Table 1. Experimental design and treatment application.

Code	Treatment	Composition	Rationale
Tctrl	Negative control	100 g soil + 10 mL sterile distilled water	Baseline nematode density without any bioactive agent
Tco	*C. odorata extract*	100 g soil + 10 mL aqueous C. odorata leaf extract	Pyrrolizidine alkaloids, terpenes, phenolics
Ttito	*T. diversifolia extract*	100 g soil + 10 mL aqueous T. diversifolia leaf extract	Sesquiterpene lactones, tagitinins, diversifolins
Ttabac	*N. tabacum extract*	100 g soil + 10 mL aqueous N. tabacum leaf extract	Nicotine, nornicotine -AChE inhibition
Tcombi	Combined extract	100 g soil + 10 mL (1:1:1 Co + Tito + Tabac)	Potential additive or synergistic nematotoxicity
Tnema	Positive control Oxamyl	100 g soil + 10 mL oxamyl (10 g L^{-1})	Synthetic reference -AChE inhibitor

Each solution (10 mL) was applied uniformly to the surface of the 100 g soil aliquot in the Petri dish. Dishes were gently agitated to promote uniform soil–extract contact, then sealed with Parafilm to limit evaporation and maintained at 28 ± 2°C in the dark for 48 hours prior to nematode extraction.

Nematode extraction and enumeration

Nematodes were extracted from treated soil using a modified Baermann funnel technique (Baermann, 1917; Hooper *et al.*, 2005). Briefly, 100 mg subsamples of treated soil were spread on poplin fabric tissues (fine-weave cotton, mesh aperture ~50 µm, non-lethal to nematodes) placed in funnels filled with tap water such that the soil was in contact with but not submerged under the water surface. Assemblies were incubated in the dark at 28 ± 2°C for 48 hours, allowing motile nematodes to migrate actively downward through the fabric into the water column. After incubation, fabric tissues were removed, the collected water filtered through a 25 µm mesh sieve, and nematode suspensions rinsed with 100 mL of tap water and recovered in glass vials. Nematode population density was determined by enumerating organisms from a 1 mL aliquot of the recovered suspension deposited onto a Malassez counting chamber (Malassez, 1874). The chamber volume is defined as:

$$V = l \times L \times h = 0.2 \times 2.0 \times 2.5 \text{ mm} = 1.0 \text{ mm}^3$$

per counting unit.

The mean count from 10 randomly selected chamber units was used to calculate the nematode concentration (C) as:

$$C \text{ (nematodes mm}^{-3}) = \bar{N}t \times 100$$

where $\bar{N}t$ is the mean nematode count per counting unit. Population density was expressed as nematodes cm^{-3} of soil.

The nematode population reduction rate (TR) for each treatment was calculated as:

$$TR\ (\%) = [(N\_ctrl - N\_treatment) / N\_ctrl] \times 100$$

where N_ctrl is the mean nematode density in the negative control and N_treatment is the mean density in the treated sample.

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) using R statistical software version 4.2.2 (R Core Team, 2022) with the RStudio interface. Assumptions of normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) were verified prior to ANOVA. When the overall ANOVA reached significance ($\alpha = 0.05$), means were compared using Tukey's Honestly Significant Difference (HSD) post-hoc test. Results are expressed as means ± standard deviation (SD) of three replicates. Figures were produced with the ggplot2 package (Wickham, 2016).

RESULTS AND DISCUSSION

Assessment of the 50 sampled pepper plants revealed a disease incidence of 100% all sampled plants exhibited visible galling symptoms on root systems. Gall severity uniformly reached index 5 on the Taylor and Sasser (1978) scale, indicating near-total root system destruction with more than 100 galls per plant. This severe infestation level confirms the suitability of the experimental site for nematode bioassay purposes, providing a naturally high and homogeneous nematode inoculum pressure in the rhizospheric soil. The mean nematode population density in untreated soil (negative control) was 400 nematodes cm^{-3}, consistent with high-infestation rhizospheric conditions reported for Meloidogyne-infested pepper crops in West Africa (Onkendi *et al.,* 2014). Following treatment application and 48 h extraction, nematode densities were significantly reduced in all botanical and synthetic nematicide treatments relative to the negative control (one-way ANOVA, $F_{5,12} = 11.978$, $p = 0.0002516 < 0.05$; Table 1, Figure 1).

The lowest nematode density among botanical treatments was recorded in soil treated with the combined extract (Tcombi), at approximately 26.7 nematodes cm^{-3}, corresponding to a population reduction of 93.3%. The positive control (oxamyl) achieved the highest reduction at 96.7%, yielding a residual density of approximately 13.2 nematodes cm^{-3}. No statistically significant difference was observed between the Tcombi treatment and the oxamyl positive control (Tukey HSD, $p > 0.05$), a critical result demonstrating near-equivalent efficacy between the combined botanical formulation and the synthetic reference compound. The Petri dish soil bioassay protocol developed in this study enables reliable quantification of nematicide activity within a five-day experimental window three days of treatment contact time followed by 48 hours of Baermann extraction compared with the three to six weeks required for standard greenhouse pot trials (Moens *et al*., 2009) or the 72-hour protocols required for in vitro larval mortality assays that necessitate purified nematode cultures (Chitwood, 2002). The use of naturally infested rhizospheric soil as the nematode source presents both an advantage and a limitation: it closely approximates field conditions and eliminates the need for axenic nematode cultures, but it precludes precise taxonomic identification and standardized inoculum quantification. Future iterations of this protocol should incorporate molecular identification of nematode genera (e.g., ITS-rDNA sequencing) to characterize the species assemblage targeted. The poplin-fabric Baermann modification used here is consistent with adaptations reported for resource-limited tropical laboratory settings (Hooper *et al*., 2005). The Malassez counting chamber provided adequate precision for the population densities observed (400 nematodes cm^{-3} in untreated soil), though the method does not discriminate between live and dead nematodes a limitation shared with all passive migration-based extraction approaches. The reduction in recovered nematode numbers therefore integrates both genuine nematicide-induced mortality and impaired motility due to sublethal toxicity, which remains a conservative but ecologically relevant endpoint for field performance prediction.

Chromolaena odorata achieved a 62.5% nematode population reduction in this study the lowest among the three individual plant species tested, but nonetheless substantially above the threshold of biological significance commonly set at 50% for preliminary screening (Oka, 2010). This result is consistent with documented pesticidal properties of this species: Paduano *et al*. (2014) identified a range of bioactive secondary metabolites in C. odorata leaf extracts, including eupatorin, demethylated eupatorin, and several pyrrolizidine alkaloids, some of which demonstrate inhibitory activity against cholinesterase enzymes relevant to nematode neurotoxicity. Chromolaena also contains high concentrations of terpene compounds notably caryophyllene and alpha-pinene that have been independently shown to affect nematode locomotion and feeding behavior (Ntalli and Menkissoglu-Spiroudi, 2011). The comparatively lower efficacy relative to N. tabacum in this study may reflect differences in the concentration and bioavailability of active compounds under aqueous extraction conditions, as lipophilic terpenes may not partition efficiently into water at room temperature. *Tithonia diversifolia* produced a 66.7% population reduction. This plant's nematicidal properties have been increasingly documented in the literature: Adeyemi *et al.* (2014) demonstrated that both dried leaf incorporation into soil and aqueous leaf extracts of *T. diversifolia* significantly suppressed *Meloidogyne incognita* populations in tomato crops, with leaf incorporation proving more effective than aqueous application. The principal bioactive compounds responsible are sesquiterpene lactones of the guaianolide and eudesmanolide types specifically tagitinin A, tagitinin

C, and diversifolin which have been shown to exert direct toxic effects on *Meloidogyne* juveniles (J2) by disrupting cuticle integrity and oxidative balance (Sotelo *et al.,* 2020). Additionally, *T. diversifolia* leaf biomass is rich in nitrogen and phosphorus, suggesting that soil amendment effects may complement direct nematotoxicity in field applications a dimension not captured by the current bioassay design.

The highest nematicidal efficacy among individual plant extracts was achieved by *N. tabacum* (83.5% reduction), a result congruent with a substantial body of evidence on tobacco's pesticidal properties. Nicotine and related pyridine alkaloids are the primary nematotoxic constituents: these compounds act as agonists of nicotinic acetylcholine receptors (nAChRs) in nematode neuromuscular junctions, inducing spastic paralysis and eventual death at concentrations achievable under typical field application rates (Chitwood, 2002; Hollingsworth *et al*., 1994). Concentrations of nicotine in dried N. tabacum leaf can reach 2–8% dry weight depending on cultivar and curing method, meaning that a 10 g L^{-1} aqueous extract potentially delivers nematotoxic concentrations within the biologically active range. Furthermore, anabasine, a secondary alkaloid in tobacco, has been independently reported to suppress *Meloidogyne* egg hatching and J2 mobility at concentrations as low as 100 ppm (Hollingsworth *et al.,* 1994). The aqueous solubility of nicotine (freely miscible with water) ensures efficient extraction and delivery to the soil matrix, which may explain the superior performance of N. tabacum relative to the other two species under the extraction protocol used.

The combined extract (Tcombi) achieved 93.3% nematode population reduction a result significantly exceeding the mean of the three individual extract efficacies (70.9%), and statistically equivalent to the synthetic nematicide oxamyl (96.7%, $p > 0.05$). This enhanced performance is consistent with the concept of phytochemical synergy documented across botanical pesticide formulations: the simultaneous presence of diverse bioactive compounds targeting different biochemical pathways in nematode physiology alkaloid-mediated neurotoxicity (nicotine), terpenoid-induced cuticle disruption (tagitinins), and polyphenol-mediated enzyme inhibition (eupatorin) is hypothesized to produce additive or true synergistic interaction effects that exceed what any single compound or extract achieves independently (Oka, 2010; Ntalli and Menkissoglu-Spiroudi, 2011).

The practical implication of this finding is considerable; a combined botanical formulation drawing on locally available, low-cost plant species may provide nematode management efficacy equivalent to synthetic nematicides without their associated toxicological and environmental costs. However, several caveats apply. First, the current bioassay does not distinguish between nematostatic (reversible paralysis) and nematicidal (lethal) effects a distinction with important implications for field performance durability. Second, the combined extract was formulated as a simple volumetric 1:1:1 mixture; optimization of the extraction ratio and concentration of each component could yield further efficacy gains. Third, the duration of residual activity in field soil conditions subject to leaching, photodegradation, and microbial breakdown was not assessed in this short-term laboratory study. Oxamyl (methyl N', N'-dimethyl-N-[(methylcarbamoyl)oxy]-1-thiooxamimidate) is a systemic carbamate nematicide that inhibits acetylcholinesterase (AChE) activity in nematode nerve tissue, causing irreversible neuromuscular paralysis and death (Chitwood, 2002). Its 96.7% reduction rate in this study is consistent with published field and laboratory efficacy data (Oka, 2010). The fact that the Tcombi treatment achieved statistically equivalent suppression (93.3%) without significant difference from oxamyl represents the most commercially and agronomically significant finding of this study, as it establishes a quantitative efficacy threshold that a multi-component botanical formulation can reach.

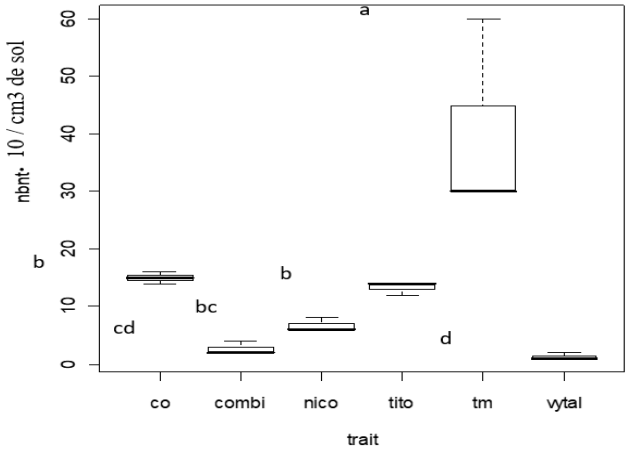

Figure 1. Efficacy of treatments (plant extracts and oxamyl) on the nematode population in infested pepper rhizosphere soil.

Co: *Chromolaena odorata* extract; combi: Combined extract; nico: *Nicotiana tabacum* extract; tito: *Tithonia diversifolia* extract; tm: Negative control; vytal: Oxamyl positive control; nbnt: nematode; trait: treatments.

Table 2. Effect of aqueous plant extracts and oxamyl on nematode population density in naturally infested pepper rhizospheric soil after 48 h treatment (means of three replicates).

Treatment	Code	Mean nematode density (cm^{-3})	Reduction rate (TR%)	Tukey group
Negative control (distilled water)	Tctrl	400.0 ± 17.32	0	a
Chromolaena odorata extract	Tco	150.0 ± 1.00	62.5	b
Tithonia diversifolia extract	Ttito	133.2 ± 1.15	66.7	b
Nicotiana tabacum extract	Ttabac	66.0 ± 1.15	83.5	bc
Combined extract (Co + Tito + Tabac)	Tcombi	26.7 ± 1.15	93.3	cd
Oxamyl positive control	Tnema	13.2 ± 0.57	96.7	d

SD: standard deviation; means followed by different lowercase letters in the Tukey group column are significantly different (Tukey HSD, $\alpha = 0.05$).

These results (Table 2) must be interpreted within the broader framework of integrated nematode management (INM). Botanical extracts are best positioned not as wholesale replacements for synthetic nematicides, but as components of diversified management strategies that include resistant cultivars, crop rotation, biological control agents (Trichoderma spp., Bacillus spp., entomopathogenic nematodes), and organic soil amendments (Coyne *et al.,* 2018). The rapid bioassay protocol developed here provides an accessible and efficient entry point for the screening phase of such integrated programs.

CONCLUSION

This study demonstrates that a Petri dish-based soil bioassay using the Baermann extraction method provides a reliable, rapid, and low-cost protocol for the preliminary screening of botanical nematicides against naturally occurring phytoparasitic nematode populations. The protocol is executable within five days using standard laboratory equipment available in most agricultural research institutions in developing countries. Among the three plant species tested, Nicotiana tabacum demonstrated the highest individual nematicidal efficacy (83.5% population reduction), consistent with the well-characterized toxicity of its alkaloid constituents toward nematode neuromuscular systems. *Tithonia diversifolia* (66.7%) and *Chromolaena odorata* (62.5%) showed significant but comparatively lower individual activity, likely reflecting differences in active compound polarity and aqueous extractability. The combined tri-plant extract achieved 93.3% nematode population reduction statistically equivalent to the synthetic reference nematicide oxamyl (96.7%) providing strong preliminary evidence for synergistic or additive interaction among the phytochemical constituents of the three species. These findings support the following recommendations for future research: (i) greenhouse and field validation of the combined botanical formulation on Meloidogyne-infested pepper and tomato crops under Ivorian agro-ecological conditions; (ii) phytochemical characterization of the aqueous extracts by HPLC-MS to identify and quantify the active compounds; (iii) optimization of extract concentration, formulation stability, and shelf life for practical agricultural application; (iv) ecotoxicological assessment of the combined formulation on non-target soil fauna; and (v) economic feasibility analysis of large-scale production and deployment within West African smallholder farming contexts.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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