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Potential of Sahelian Native Shrub Materials to Suppress the Spiral Nematode *Helicotylenchus dihystera*

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Abstract: Pearl millet (*Pennisetum glaucum*) is a drought-tolerant cereal commonly grown for grain and fodder in arid areas throughout the world. Senegalese millet fields are infested with *Helicotylenchus*. The native evergreen woody shrub *Piliostigma reticulatum* is widely distributed in sub-Saharan Africa. Its coppiced residues are used by small farmers as mulch in crop fields. The shrub's nematicidal effect on the spiral nematode *Helicotylenchus dihystera* was evaluated in a pearl millet pot experiment. The abundance of nematodes decreased by 64% after application of either leaf powder or a pulverized mixing of leaves and stems, suggesting the use of aboveground materials of *P. reticulatum* as a potential nematicide. The results show promise for use of a local resource by subsistence farmers in the Sahel. Further research is needed on application to fully develop this approach as a biopesticide.

Key words: Biological control, *Helicotylenchus dihystera*, millet intercropping, native evergreen woody shrub, nematicidal activity, *Pennisetum glaucum*, *Piliostigma reticulatum*, plant-parasitic nematode, Sahelian agrosystem management, soil organic amendment, spiral nematode, western Africa.

Pearlmillet (*Pennisetum glaucum* (L.) R. Br.) is widely cultivated by sub-Saharan small-landholders for grain and fodder because it is well adapted to drought and the low-fertility soils of the semiarid African Sahel. However, plant-parasitic nematodes have been reported in millet rhizosphere where they can alter crop growth (Villenave and Cadet, 1998; Cadet and Floret, 1999; McDonald and Nicol, 2005). *Helicotylenchus* and *Scutellonema* (both Hoplolaimidae) were recently reported by Diakhaté et al. (2013) to be highly abundant in soils of millet field in Senegal.

Management of plant-parasitic nematode is difficult. The use of chemical nematicides raises environmental concerns, and their cost remains prohibitive for African small-landholders. Plant-parasitic nematodes control in cereals has depended largely on use of crop rotation and a limited number of resistant cultivars (Timper and Wilson, 2006; Timper et al., 2007). Local natural resources can also be used to manage nematode pests. Bioactive products derived from Neem tree (*Azadirachta indica* A. Juss) are suppressive on plant-parasitic nematodes (Akhtar and Malik, 2000; Jonathan and Uma Maheswari, 2008). Furthermore, evidence that plant-parasitic nematodes are more susceptible to plant-derived nematicides than beneficial nematodes is increasing (Bar-Eyal et al., 2006). Thus, biological control with a locally available plant resource could provide an advantage over commercial nematicides by protecting nontarget and nematode-antagonistic soil organisms, including predatory nematodes (Oka et al., 2001; Bar-Eyal et al., 2006).

Piliostigma reticulatum (DC.) Hochst is a native evergreen woody shrub that commonly coexists with staple food crops in farmers' fields of sub-Saharan Africa (Lufafa et al., 2008; Lahmar et al., 2012). Intercropping millet with this shrub and using its coppiced residues as a mulch has shown positive impacts on millet growth and yield (Dossa et al., 2009, 2013). Diakhaté et al. (2013) reported that this intercropping system had positive effects on free-living nematode communities while suppressing plant-parasitic nematodes in soil, mainly Hoplolaimidae family. Several studies have suggested that organic amendments could be used to control plant-parasitic nematodes with mulching preferred over incorporation into soil (e.g., Singh and Sittaramiah, 1970; Akhtar and Malik, 2000; Stirling et al., 2011).

Some phenolic compounds in *P. reticulatum* materials (Babajide et al., 2008; Dossa et al., 2009) were known as natural nematicides for other species (Chitwood, 2002).

The perennial shrub *P. reticulatum* is widely distributed throughout the Sudano-Sahelian savanna from Senegal to Sudan (Lufafa et al., 2008; Hernandez et al., 2015) and is well known for its antibacterial and antifungal properties (Yelemou et al., 2007; Babajide et al., 2008). Although Diakhaté et al. (2013) reported in situ shrub control of nematodes in millet fields, nematicidal properties of its aboveground residue has not been investigated. We hypothesized that the shrub material has a suppressive effect on *Helicotylenchus dihystera* (Cobb) Sher, the main plant-parasitic nematode found in millet systems, and that *P. reticulatum* can serve as a resource for nematode pest management by local farmers.

Materials and Methods

Preparation of inoculum of H. dihystera: A fine sandy alfisol (Soil Management Support Services, 1985) was

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sampled at 0- to 10-cm depth in a non-fertilized millet monoculture of an experimental station located at Nioro-du-Rip (southern region of the Senegalese Peanut Basin). It contained 3.0 mg C/g soil and 0.26 mg N/g soil of total carbon and nitrogen, respectively, and 1.5 μg $N\text{-}NO_3^-$/g soil and 3.2 μg $N\text{-}NH_4^+$/g soil as mineral N, with a pH in water of 5.2 (Diakhaté et al., 2013). The soil was passed through a 2-mm mesh sieve and autoclaved before wetting at 60% of the soil water holding capacity (WHC). The sterilized soil was placed in 500-ml plots and millet (*Pennisetum glaucum* (L.) Br.) seeds were planted. Two weeks later, another soil sampling was conducted by collecting soil from the root system of millet growing in the field. Nematodes were immediately extracted using Seinhorst's elutriation technique (Seinhorst, 1962). The root-feeder *H. dihystera* was selected as the candidate for the monospecific inoculum because of (i) its predominance in the natural assemblage of the plant-parasitic nematodes in the millet monoculture (Diakhaté et al., 2013) and (ii) its life strategy in soil. The nematodes were identified under a dissecting microscope as *H. dihystera* on the basis of their morphological characteristics and were inoculated (ca. 100 mature individuals/100 g soil) into the pots to allow for a pure population to grow. After 4 wk, the pots were destroyed, and *H. dihystera* extracted from soil by elutriation (Seinhorst, 1962) and inoculated again on new plants in pots. This operation was repeated until enough nematodes were obtained.

Preliminary in vitro test: Fresh leaves of *P. reticulatum* collected at the experimental station of Nioro-du-Rip were coarsely chopped and dispersed in distilled water (1/3 as w/v ratio) for 24 hr at room temperature. After separation of the crude plant extract on a 30-μm nylon sieve, the supernatant was centrifuged and filtered through Whatman No.1 paper filter. Pure populations of *H. dihystera* were introduced in 5-ml Eppendorf tubes (ca. 140 individuals per tube; $n = 4$) containing 1 ml of either the leaf extract or distilled water (the latter serving as a control). Living and active nematodes were counted using a light microscope after 24 hr exposure in the dark at 27°C. The spiral nematodes were considered dead (or completely paralyzed) when no movement was observed during 2 sec even after mechanical prodding. The test was repeated twice.

Experimental setup: Shrub branches were collected from living stands (at least 2 m in diameter) at Nioro-du-Rip. On several occasions, fresh leaves were separated from the stem and stored independently. Both coppiced branches (leaf + stem) and the separated leaves were individually washed, dried at 50°C over 48 hr, mechanically pulverized (1-mm size) and stored at room temperature in sterilized flasks before use. Then the trial was repeated twice as the following. Inoculation of 3-wk-old millet plants grown in a 500-ml pot containing 350 g of steam-sterilized soil was conducted by pouring 2 ml water containing 845 ± 30 spiral nematodes in small pits at the soil surface. Four days after inoculation, nematodes were extracted from sacrificed two pots and counted to verify the population density. Then, shrub materials (leaves or leaf + stem) were applied evenly on the soil surface at a rate of 2% (w/w). One series received no plant material and served as a nematode-infested control. Each treatment consisted of six replicates that were maintained in a completely randomized experimental design at 27°C in a plant growth chamber. Each pot was regularly watered with distilled water to maintain a 60% WHC in soil. Forty five days after inoculation and soil amendment, the plants were removed from pots and aboveground biomasses weighed while nematodes were extracted from four pots per treatment (Seinhorst, 1962). Nematodes were counted using a light microscope. The two remaining pots per treatment were used to colorimetrically determine soil mineral N contents in KCl 1 M extracts by flow injection analysis (Bremner, 1965).

Statistical analysis: The experiment was conducted twice. As the same trends were observed in the two trials (ANOVA on nematode abundance; $F = 0.052$; $P = 0.822$), the data were pooled (eight replicates/treatment), analyzed using one-way analysis of variance (ANOVA) and Fisher's least significant difference tests were calculated to identify significant treatment effect at a probability levels of $P \leq 0.05$ using XLSTAT software (v2010 AddinSoft®).

Results and Discussion

The in vitro preliminary tests revealed that all nematodes were killed or completely paralyzed by the 24-hr immersion in the leaf extract of *P. reticulatum* while 86% remained active in water-only control treatment. Exposure of the nematodes to an aqueous extract in vitro is a common and rapid test for evaluating the potential of soil amendments to be suppressive (Oka et al., 2001).

In the pot experiment, nematode population density was affected by the treatments ($F = 78$; $P < 0.0001$). Where no amendment was added, the populations of *H. dihystera* increased from 845 (± 30) individuals/100 g soil to 8,555 (± 1,120) individuals after 45 days (Table 1). In pots where soil was amended with the shrub materials, the abundance of *H. dihystera* was 3,089 (± 910) and 3,072 (± 997) individuals/100 g soil in leaf and leaf+stem treatments, respectively (Table 1). The addition of both shrub materials to soil equally resulted in a 64% decrease in nematode abundance after 45 days. Diakhaté et al. (2013) reported the suppression of plant-parasitic nematodes in a Senegalese agrosystem where millet was intercropped with *P. reticulatum* and shrub coppicing residues used as mulch.

The aboveground biomass of young millet decreased under *P. reticulatum* amendments compared to the

Table 1. Effect of soil amendment with *P. reticulatum* materials on *H. dihystera* abundance, millet aboveground biomass and soil mineral N contents after 45 d.

Treatment[a]	Nematode abundance[b] (Individuals/100 g soil)	Millet biomass (g/pot)	Soil nitrate (μg N-NO_3^-/g soil)	Soil ammonium (μg N-NH_4^+/g soil)
Control	8,555 a	0.26 a	0.54 a	1.67 a
Leaf	3,089 a	0.16 b	3.30 c	1.95 b
Leaf + stem	3,072 b	0.05 c	2.94 b	2.33 c

[a] The control received no amendment.
[b] The initial nematode population was 845 (± 30) individuals/100 g soil.
[c] Data followed by the same letters in each column are not significantly different ($P \leq 0.05$) according to least significant difference's test; $n = 8$ except for soil nitrate and ammonium where $n = 4$.

infested control while the plants grown in soil amended with the leaf + stem powder were more stunted after 45 d than with leaf application (Table 1). The nitrate and ammonium contents of the infested control were 0.54 μg N-NO_3^-/g soil and 1.67 μg N-NH_4^+/g soil, respectively, after 45 d. Mineral N increased in soils amended with both shrub materials with significant differences observed between leaf and leaf + stem treatments while nitrate became more abundant than ammonium (Table 1). A potential undesirable feature of plant-derived nematicidal compounds is allelopathy or phytotoxicity toward plants, especially in the early growth stages (Li et al., 2010). One such compound of litter is polyphenol, which affects nutrient cycling by serving as a substrate for respiration and by altering nitrogen availability (Cadisch and Giller, 1997). Powders of *P. reticulatum* leaf or branch materials at the rates of application used in our study caused reduced aboveground biomass of the young millet plants, despite a non-limited N mineral content in the amended soils. Similar effects were observed by Oka et al. (2012) who reported that leaf powder of *Myrtus communis* or *Inula viscosa*, amended to soils at relatively high rates had nematicidal properties but at the same time reduced shoot growth of tomatoes. Dossa et al. (2009) measured chemical properties of *P. reticulatum* materials collected at the same site as we did and demonstrated that leaves have lower levels of polyphenols than the whole branches (leaf + stem). As both powders derived from shrub aboveground materials were equally effective in suppressing plant-parasitic nematodes, leaves would be preferred over leaf + stem. In summary, results of the present study indicate that *P. reticulatum* litter has nematicidal potential against *H. dihystera* which is a dominant nematode pest in agrosystems of western Africa. Additional studies are required to determine the optimal rates of application that do not negatively affect plants but still maintain suppression of nematode pests.

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