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Research Article

Defense Mechanisms of Sweet Potato Varieties (*Ipomoea batatas* L. [Lam]) Enhanced by Neem Seed Extract Against Root-knot Nematodes and *Fusarium* Wilt

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Abstract

Sweet potato crops are severely threatened by nematodes of the genus *Meloidogyne* spp. and *Fusarium* sp. fungi, which are responsible for *Fusarium* wilt. Nematodes cause root and tuber deformities, as well as wilting of stems and leaves, while *Fusarium* wilt leads to rot, necrosis, and wilting. These two pests contribute to a significant reduction in yields and substantial losses of production. Control measures primarily rely on the use of chemicals, whose excessive use poses a threat to the environment and human health. This study aims to assess the tolerance of four sweet potato varieties treated with aqueous neem seed extract against combined attacks from nematodes and *Fusarium spp*. A completely randomized block design with four sweet potato varieties (V1: white variety from the center region (togologo); V2: white variety from Adamawa region (lambadidi); V3: Improved Yellow variety (IRAD 1112); V4: Yellow variety from the center region (Bété)) and four treatments (T0: non-inoculated varieties; T1: varieties inoculated with nematodes + *Fusarium*; T2: varieties inoculated with nematodes + *Fusarium* then treated with neem aqueous extract; T3: varieties inoculated with nematodes + *Fusarium* then treated with synthetic pesticides) was used in a greenhouse. Agro-morphological, epidemiological parameters, and biochemical resistance markers were evaluated. The results revealed that treatment with neem extract (T2) reduced severity rates by 60 to 80% in the different growing bags compared to treatment (T0), followed by treatment (T3). The highest yields were obtained in treatments T1 and T2 across all varieties. The Adamawa White variety (lambadidi) (V2) and the IRAD 1112 variety (V3) exhibited the highest yields with up to 7 t/ha in T3 treatment. The content of phenolic compounds, proteins, and the enzymatic activity of peroxidase were higher in sweet potato plants inoculated with *Fusarium* and treated with neem aqueous extract. Neem treatment showed strong protective potential in combating fungal diseases and nematodes affecting sweet potato.

Keywords

Sweet Potato Disease, Neem Aqueous Extract, *Fusarium* sp., *Meloidogyne* spp., Biochemical Markers

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1. Introduction

Sweet potato (*Ipomoea batatas* L. [Lam]) is a perennial herbaceous plant belonging to the Convolvulaceae family. It is believed to be native to South America [1]. and thrives in both tropical and subtropical regions [2] as well as in some warm temperate areas where it is cultivated as an annual plant [3]. Cultivated on approximately 9 million hectares across five continents, the global annual production of sweet potatoes is estimated at nearly 104 million tons, making it one of the most widely cultivated tubers in the world [4]. China is by far the largest producer of sweet potatoes, with an annual production of around 49 million tons, followed by Malawi, Tanzania, and Nigeria, producing approximately 7 million, 4 million, and 3 million tons per year, respectively. In Cameroon, the annual production of sweet potatoes is estimated at 411,489.4 tons [4]. Sweet potato has become a globally important crop with diverse applications in food, medicine, and the agro-food industry. The leaves of sweet potato are used for both human and animal consumption. Its tubers are rich in anthocyanins, antioxidants, β-carotene, and nutrients [5]. Consumption of sweet potatoes has favorable effects on blood sugar regulation [6]. Its production and marketing provide a source of income for farmers in Africa [7]. Its tubers are also used for the production of starch and bioethanol [8]. Furthermore, due to its adaptability, sweet potato can be cultivated in a variety of agroecosystems, making it a promising crop for various regions, including sub-Saharan Africa. However, despite three-quarters of the land in sub-Saharan Africa being arable, agricultural production remains very low [4]. This situation is due to several factors, including pest attacks, the absence of high-performing and pest-resistant varieties, lack of healthy seeds, drought, soil poverty, and soil salinization [9]. Pest damage to sweet potatoes includes leaf destruction, wilting of stems, and tuber rot, leading to reduced yields. In sweet potatoes, nematodes of the genus *Meloidogyne* spp. attack both roots and tubers, causing swellings or protuberances of various shapes. If the initial nematode population is high, they lead to excessive production of lateral roots associated with vigorous growth [10]. Hypersensitive and resistant plants exhibit root tip necrosis, while sensitive cultivars develop generalized necrosis throughout the root [11]. Infected tubers crack at maturity, facilitating attacks by secondary organisms and leading to subsequent rot [12]. Upon cross-section observation of infected tubers, female nematodes are visible and are typically surrounded by necrotic cells. The reduced root growth of attacked plants is accompanied by symptoms such as yellowing, stunted growth of aerial parts, and wilting, particularly during hot periods of the day [13]. The weakened plant is exposed to diseases. Vascular *Fusarium* wilt caused by *Fusarium oxysporum* infects sweet potatoes through natural openings or wounds. This fungus forms microconidia that circulate in the plant through the xylem, blocking vessels and leading to yellowing of leaves, the appearance of wrinkles around veins, wilting, and, in severe cases, plant death [14]. To reduce pest attacks, sweet potato producers in Cameroon primarily use synthetic pesticides, despite their harmful consequences [15]. The first organisms affected are insects, with an estimated extinction rate of 1% to 2% per year [16]. Acute and chronic effects manifest in agricultural workers through cancer pathologies, neurological disorders, and reproductive problems [16]. To reduce the abusive use of synthetic pesticides commonly employed to control pests, it is crucial to encourage alternative control methods such as cultural practices and biological control based on the use of tolerant varieties and pesticide potential plants. Plants possess various mechanisms to counter the harmful effects of pathogens and pests. Biochemical defense involving secondary metabolites can act directly or indirectly on pests by activating non-enzymatic (phenols, flavonoids, glutathione, etc.) and enzymatic (PR proteins, peroxidases, or polyphenol-oxidases, etc.) antioxidant responses [17, 18]. Bio-pesticide from plants, such as neem (*Azadirachta indica*), have proven effective in crop protection. Previous studies reported that raw extracts prepared from the neem leaves, stems, flowers, or seeds have fungicidal, bactericidal, insecticidal, and nematicidal properties [19-22]. In addition, plant extract as biopesticide are affordable and biodegradable [23]. Thus, the present study was carried out to evaluate the tolerance of four sweet potato varieties treated with neem aqueous extract against nematodes and *Fusarium* wilt.

2. Materials and Methods

2.1. Isolation of *Fusarium*

Stems and roots exhibiting typical symptoms of the disease were collected from an experimental field in Yaounde, Nkoabang (3°40'60" N latitude and 11°25'0" E longitude) and brought to the laboratory of phytopathology at the University of Yaounde I. The stems or roots were excised into a length of approximately 15 mm and placed in Petri dishes containing solidified Potato Dextrose Agar (PDA) medium and incubated for 6 to 7 days [24]. Microscopic observations of the conidia were then conducted to confirm the identity of the pathogen. Once the identity was confirmed, mycelial disks were taken from the Petri dish and transferred to a new culture medium for fungal growth. This final step was repeated several times to obtain pure fungal strains.

2.2. Isolation of Nematodes

Nematodes was extracted from infected soil based on method described by Mendoza-de Gives [25]. The rhizosphere soil of infected plants was collected, air-dried at room temperature, then crumbled and sieved. The infected organ

fragments were cleaned and then ground, placed in sieves covered with Whatman paper and muslin cloth. The samples were saturated with water, supplemented daily with sterile water while gently shaking the sieves to promote aeration. After three days, the filtrate obtained was allowed to settle. The upper part was removed, and the bottom rich in nematodes was retained for microscopic observation. Nematode identification was based on a comparative observation of their morphology [26].

2.3. Preparation of the Extract in the Laboratory

Mature neem seeds were crushed and stored at laboratory temperature for three days; The obtained kernels were weighed using a scale and then ground. The paste obtained after grinding the seeds was wrapped in muslin cloth and directly immersed in distilled water for at least 12 hours. The next day, the cloth containing the paste was gently squeezed to extract the maximum product [27]. The aqueous extract thus prepared was used directly.

2.4. Phytochemical Screening of the Neem Seed Extract

The major families of secondary metabolites were sought in the plant following the classical characterization protocols described by Edeoga et al. [28]; Sahuvinod et al. [29]. Tannins and polyphenols were identified using the FeCl3 test and Stiasny's reagent; flavonoids by the cyanidine reaction; saponins by the foam test; quinones by the Bortr äger test; terpenes and steroids by the Liebermann-Burchard test; and alkaloids by the Mayer and Dragendorff tests [30].

2.5. Inoculation of Nematodes and *Fusarium* Conidial Suspension

From the obtained nematodes, a suspension of 20 nematodes/ml was introduced into bags containing three-week-old sweet potato plants, which were grown from 15 cm stem fragments. The nematodes were counted under an optical microscope by taking aliquots of the suspension using a pipette and placing them on a counting chamber made of Plexiglas. Volumes of 1 to 4 ml of the suspension were used for counting [31]. In addition, the conidial suspension of *Fusarium oxysporum* was prepared from a pure culture of isolates aged 10 days contained in Petri dishes. The mycelium was collected, homogenized in sterile distilled water, and the resulting suspension was calibrated using a Mallassez cell to obtain 2 x 10 ^{3}conidia/ml [32].

2.6. Experimental Design

Experimentation was conducted in a greenhouse at the Biotechnology Center of the University of Yaound éI, following a completely randomized block design, replicated three times. Each block consisted of four varieties (V1: White from the Center (*togologo*); V2: White from Adamawa (*lambadidi*); V3: Improved Yellow (IRAD 1112); V4: Yellow from the Center (*B á é*)) and four treatments (T0: non-inoculated varieties; T1: varieties inoculated with a suspension of 20 nematodes/ml and a conidial suspension of 2 x 10 ^{3}conidia/ml of *Fusarium*; T2: varieties inoculated with a suspension of 20 nematodes/ml and a conidial suspension of 2 x 10 ^{3}conidia/ml of *Fusarium*, then treated with neem aqueous extract; T3: varieties inoculated with a suspension of 20 nematodes/ml and a conidial suspension of 2 x 10 ^{3}conidia/ml of *Fusarium*, then treated with synthetic pesticides). A mixture of sterilized black soil and medium sand in a 2:1 ratio was used as a substrate. This was placed in 5 kg bags. In each bag, two defoliated stolons of 30 cm, with the lower end having spent 24 hours in either a *Fusarium* solution or a nematode solution, were introduced into the substrate.

2.7. Evaluation of Agro-Morphological and Epidemiological Parameters

Observations were made on the number of leaves and the size of the branching emerging from the bud of the last node at the upper end at 5, 7, and 9 weeks after planting (WAP).

Epidemiological parameters regarding incidence and severity were evaluated according to the formula by Tchoumahov and Zahanova [33]. The incidence of plants affected by nematodes and the disease was assessed using the following formula:

$$I\ (\%) = n/N \times 100$$

Where: I is the incidence of the disease, n is the number of plants affected by nematodes and *Fusarium*, N is the total number of sampled plants.

The degree of infection or severity of the plants by nematodes and the disease was evaluated on 30 plants per block, by estimating the leaf area occupied by disease symptoms using the following formula:

$$S\ (\%) = \sum (ab)\ /N \times 100$$

Where: Σ (ab) is the sum of the products of the number of plants affected by nematodes and the disease (a) and the degree of infection (b) given in %, and N is the number of plants affected by nematodes and the disease.

2.8. Evaluation of Some Biochemical Parameters

Symptomatic leaves were randomly collected from the different subplots and treatments. Leaf samples from the four sweet potato varieties were brought to the laboratory to determine the total phenol content, total protein content, and the

activity of two antioxidant enzymes: peroxidases (POX) and phenylalanine ammonia-lyase (PAL). Each test was conducted in triplicate.

2.8.1. Extraction of Total Phenolic Compounds

The leaves of the sampled sweet potato varieties were ground. One gram of this powder was mixed with a 0.1N hydrochloric acid (HCl) solution and then incubated at room temperature for 30 minutes. The resulting mixture was centrifuged for 30 minutes at 6000 g, and the supernatant collected constituted the crude extract of the phenolic compounds [34].

2.8.2. Dosage of Total Phenolic Compounds

The obtained crude extract was quantified according to the method of Singleton and Rossi [35], modified. In an alkaline medium and at high temperature, the Folin-Ciocalteu reagent is reduced during the oxidation of phenolic compounds to a mixture of blue tungsten and molybdenum oxides. In a test tube, 10 µL of the crude extract, 1990 µL of distilled water, 250 µL of Folin-Ciocalteu reagent, and 500 µL of 20% Na_2CO_3 were added. The resulting mixture was incubated at 40 ℃ for 30 minutes. The absorbance was measured using a spectrophotometer (UV-1605; UV-visible SPECTROPHOTOMETER SHIMADZU) at 760 nm against a blank in which the extract was replaced by distilled water. The phenolic compound content was expressed in mg/g of fresh weight (FW).

2.8.3. Extraction and Quantification of Total Soluble Proteins

(i). Extraction of Total Soluble Proteins

The extraction of proteins was performed according to the method established by Tarafdar and Marschner [36], modified. One gram of fresh leaves was ground in a mortar placed on melting ice, in the presence of 5 ml of sodium phosphate buffer (0.4 mol/L; pH 5) and one gram of sterile sand. The obtained extract was centrifuged at 5000 rpm for 10 minutes. The supernatant containing the crude extract was collected, stored in Eppendorf tubes, and kept at -20 ℃.

(ii). Quantification of Total Soluble Proteins

The quantification of total soluble proteins in the obtained extracts was carried out using the protocol of Bradford [37], which relies on the binding of the Coomassie Blue dye to proteins. This method uses BSA (Bovine Serum Albumin, 1 mg/ml) as a standard protein. To 70 µL of the obtained extract, 500 µL of phosphate buffer, 450 µL of distilled water, and 2000 µL of Bradford reagent wereadded. The values obtained after measurement using a spectrophotometer at 595 nm (UV-1605; UV-visible SPECTROPHOTOMETER SHIMADZU) against a blank in which the extract was replaced by distilled water were compared to the values expressed by a calibration curve (Y = 0.0382, R^2= 0.9935).

2.8.4. Activity of Antioxidant Enzymes

(i). Activity of Peroxidase (POX EC 1.11.1.7)

The enzymatic activity of peroxidase in the proteins was measured according to the method of Rodriguez and Sanchez [38]. The reaction medium contained: 1 ml of phosphate-citrate buffer (0.5 M, pH 4.6); 1 ml of Guaiacol (40 mM); 0.5 ml of H2O2 (26 mM); and 40 µL of protein extract. The mixture was incubated at room temperature for 5 minutes. The appearance of tetraguaiacol was monitored by spectrophotometry (UV-1605; UV-visible SPECTROPHOTOMETER SHIMADZU) at 470 nm, corresponding to the maximum absorption wavelength of tetraguaiacol. The activity of POX is expressed in ΔA 470/min/g of protein.

(ii). Activity of Phenylalanine Ammonia-Lyase (PAL: EC 4.3.1.5)

The method of Van Kemmenn and Broumer [39] with some modifications was used to evaluate the activity of Phenylalanine ammonia-lyase. A mixture of 50 µL of phosphate buffer (0.1 M, pH 7), 50 µL of phenylalanine (50 mM), and 50 µL of enzymatic extract was incubated at 37 ℃ for 30 minutes. The reaction was stopped by adding 0.5 mL of 5N HCl, and the absorbance was measured by spectrophotometry (UV-1605; UV-visible SPECTROPHOTOMETER SHIMADZU) at 290 nm against a blank. The results were expressed in ΔA/min/g of protein.

2.9. Data Analysis

The collected data were entered and processed in Excel 2013 for each treatment and each variety. A one-way and two-way analysis of variance (ANOVA) was performed using R software version 3.5.1. Differences between means were compared using Tukey's test at ($P \leq 0.05$) when the normality of the data (Shapiro-Wilk test; $P > 0.05$) and the homogeneity of variance (Levene's test; $P > 0.05$) were verified.

3. Results

3.1. Identification of *Fusarium* and Nematodes

Symptoms in sweet potatoes, such as root galls and cracking of storage roots, provided an excellent visual diagnosis of infection by root-knot nematodes. After the extraction of nematodes, microscopic observation revealed nematodes of the genus Meloidogyne (Figure 1) and characteristic conidia of vascular *Fusarium*.

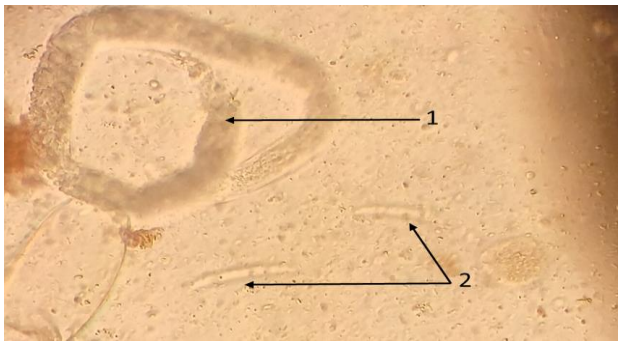

Figure 1. *Microscopic appearance of nematodes (1) and conidia of Fusarium oxysporum (2) obtained after extraction.*

3.2. Phytochemical Screening

The phytochemical screening of neem seed extract revealed the presence of several compounds belonging to various chemical classes, including alkaloids, saponins, flavonoids, sugars, and sterols, with alkaloids and flavonoids being the most abundant (Table 1).

Table 1. *Phytochemical screening of the aqueous extract of neem seeds.*

Constituents	Observations
Alkaloids	+++
Saponins	+
Tannins	-
Flavonoids	+++
Sugars	+
Phenols	-
Steroids	++
Anthraquinones	-
Glycosides	-
Terpenes	-
Extraction efficiency	.....

(-) absent; (+) trace; (++) weakly present; (+++) strongly present

3.3. Effect of Treatments and Variety on the Number of Leaves of Sweet Potato Varieties in Greenhouse

The number of leaves of sweet potato varieties under treatments over time is presented in Table 2. No significant differences were recorded between treatments and between varieties ($P > 0.001$). The effects of variety, treatment, and their interaction are non-significant, except for the treatment effect ($P = 0.003271$) during the last observation period (9 WAP). During this period, plots treated with neem extracts (T2) exhibited the highest number of leaves (15.67 ±1.61) in variety V2, compared to plots T0 and T1, which recorded the lowest number of leaves in variety V4 (9.33 ±1.15 and 9.83 ± 0.29, respectively for plots T0 and T1).

Table 2. *Evolution of the number of leaves under the influence of treatments over time.*

Varieties	Treatments	5 WAP	7 WAP	9 WAP
V1	T0	4.50 ± 1.32[a]	7.00 ± 1.00 a	14.00 ± 1.00 ab
	T1	4.33 ± 1.44 a	7.00 ± 1.32 a	10.67 ± 1.04 ab
	T2	3.67 ± 1.04 a	6.17 ± 1.04 a	13.67 ± 3.51 ab
	T3	3.50 ± 0.50 a	6.33 ± 0.76 a	11.00 ± 1.73ab
V2	T0	3.83 ± 0.29 a	6.83 ± 0.29 a	13.17 ± 0.76 ab
	T1	4.17 ± 0.76 a	6.67 ± 0.76 a	11.67 ± 2.31 ab
	T2	3.83 ± 1.04 a	6.83 ± 1.04 a	15.67 ± 1.61 a
	T3	4.00 ± 0.87 a	7.00 ± 0.50 a	12.00 ± 1.32 ab
V3	T0	3.50 ± 0.50 a	6.17 ± 0.58 a	10.50 ± 1.32 ab
	T1	4.50 ± 1.80 a	7.50 ± 1.32 a	12.50 ± 2.50 ab
	T2	3.50 ± 0.50 a	6.33 ± 1.04 a	13.33 ± 2.47 ab
	T3	3.67 ± 1.26 a	6.17 ± 1.04 a	13.00 ± 3.61 ab
V4	T0	3.50 ± 0.00 a	7.00 ± 0.50 a	9.33 ± 1.15 b

Varieties	Treatments	5 WAP	7 WAP	9 WAP
	T1	3.67 ± 0.29 a	6.50 ± 0.50 a	9.83 ± 0.29 b
	T2	3.67 ± 0.76 a	6.17 ± 1.15 a	14.00 ± 1.50 ab
	T3	4.83 ± 0.76 a	7.33 ± 0.76 a	11.83 ± 0.76 ab
P(>F) V		0.8634 ns	0.9541 ns	0.144593 ns
P(>F) T		0.5272 ns	0.6048 ns	0.003271 **
P(>F) V*T		0.5869 ns	0.6403 ns	0.188324 ns

Numbers in the same column followed by the same letter are not significantly different at a 5% degree of freedom threshold. WAS: weeks after sowing; T0: non-inoculated varieties; T1: varieties inoculated with nematodes + Fusarium; T2: varieties inoculated with nematodes + Fusarium then treated with aqueous neem extract; T3: varieties inoculated with nematodes + Fusarium then treated with synthetic pesticides. (V1: White of the Center (togologo); V2: White variety of Adamaoua (lambadidi); V3: Improved Yellow variety (IRAD 1112); V4: Yellow variety of the Center (Bété))

3.4. Effect of Treatments and Variety on the Number of Branches in Sweet Potato

The number of branches was not significantly (P > 0.05) affected by the applied treatments and variety throughout the observation period. However, the effect of variety was significant (P < 0.05) during 7 and 9 WAP. The number of branches varied from 3.63 ± 0.33 to 5.13 ± 0.33, with the lowest numbers found in varieties V1 and V4 (Table 3).

3.5. Effects of Treatments and Variety on the Incidence of *Fusarium* Wilt in Sweet Potato

Observation of the evolution of infestation rates of plots by the disease shows variation over time and according to varieties. Analysis of variance indicates significant differences (P < 0.05) between the incidence values obtained in the plots, except in the last week of observation where all plots have an incidence of 100% (Table 4). The effects of variety, treatment, and the variety-treatment interaction are significant (P < 0.05) for the first two observation periods (5 and 7 SAS). The incidences of *Fusarium* wilt varied from 0 to 33.33% during 5 SAS, while they ranged from 33.33% to 100% during 7 SAS. The plots inoculated with *Fusarium* and untreated (T1) exhibited the highest expansion rates.

Table 3. *Effect of Treatments and Variety on the Diameter of Sweet Potato Internodes.*

Varieties	Treatments	5 WAP	7 WAP	9 WAP
V1	T0	1.42 ± 0.52 a	2.17 ± 0.29 a	4.17 ± 1.26 a
	T1	1.67 ± 0.29 a	2.83 ± 0.63 a	3.83 ± 0.69 a
	T2	1.58 ± 0.14 a	2.50 ± 0.43 a	3.88 ± 0.45 a
	T3	1.83 ± 0.29 a	2.75 ± 0.25 a	3.79 ± 0.85 a
V2	T0	1.75 ± 0.43 a	3.25 ± 0.50 a	5.00 ± 0.25 a
	T1	1.58 ± 0.14 a	3.08 ± 0.72 a	5.13 ± 0.33 a
	T2	2.00 ± 0.00 a	3.08 ± 0.38 a	4.63 ± 0.13 a
	T3	2.00 ± 0.25 a	3.33 ± 0.14 a	4.88 ± 0.45 a
V3	T0	2.08 ± 0.88 a	3.00 ± 0.00 a	4.08 ± 0.14 a
	T1	1.92 ± 0.38 a	3.08 ± 0.63 a	4.13 ± 0.22 a
	T2	2.04 ± 0.19 a	2.67 ± 0.38 a	3.88 ± 0.33 a
	T3	1.83 ± 0.14 a	2.83 ± 0.14 a	3.83 ± 0.79 a

Varieties	Treatments	5 WAP	7 WAP	9 WAP
V4	T0	2.00 ± 0.00 a	2.83 ± 0.14 a	3.67 ± 0.29 a
	T1	2.08 ± 0.38 a	2.58 ± 0.29 a	3.63 ± 0.33 a
	T2	1.79 ± 0.26 a	2.67 ± 0.38 a	3.88 ± 0.45 a
	T3	1.75 ± 0.00 a	3.00 ± 0.00 a	4.33 ± 0.26 a
P(>F) V		0.1027 ns	0.004473 **	<0.001 ***
P(>F) T		0.9813 ns	0.4537 ns	0.8760 ns
P(>F) V*T		0.5321 ns	0.6008 ns	0.7736 ns

Numbers in the same column followed by the same letter are not significantly different at a 5% degree of freedom threshold. WAS: weeks after sowing; T0: non-inoculated varieties; T1: varieties inoculated with nematodes + Fusarium; T2: varieties inoculated with nematodes + Fusarium then treated with aqueous neem extract; T3: varieties inoculated with nematodes + Fusarium then treated with synthetic pesticides. (V1: White of the Center (togologo); V2: White variety of Adamaoua (lambadidi); V3: Improved Yellow variety (IRAD 1112); V4: Yellow variety of the Center (Bété)).

Table 4. *Effect of Treatments on the Incidence of Fusarium Wilt in Sweet Potato.*

Varieties	Treatments	5 WAP	7 WAP	9 WAP
V1	T0	0.0 ± 0.0 b	16.7 ± 7.6 d	100.0 ± 0.0 a
	T1	33.3 ± 8.4 a	100.0 ± 10.0 a	100.0 ± 0.0 a
	T2	0.0 ± 0.0 b	41.7 ± 7.6 cd	100.0 ± 0.0 a
	T3	0.0 ± 0.0 b	33.3 ± 10.1 cd	100.0 ± 0.0 a
V2	T0	0.0 ± 0.0 b	16.7 ± 5.5 d	100.0 ± 0.0 a
	T1	8.3 ± 1.4 b	58.3 ± 10.4 bc	100.0 ± 0.0 a
	T2	0.0 ± 0.0 b	33.3 ± 14.4 cd	100.0 ± 0.0 a
	T3	0.0 ± 0.0 b	33.3 ± 9.1 cd	100.0 ± 0.0 a
V3	T0	0.0 ± 0.0 b	18.3 ± 5.8 d	100.0 ± 0.0 a
	T1	25.0 ± 5.0 a	83.3 ± 15.3 ab	100.0 ± 0.0 a
	T2	0.0 ± 0.0 b	33.3 ± 9.7 cd	100.0 ± 0.0 a
	T3	0.0 ± 0.0 b	33.3 ± 8.5 cd	100.0 ± 0.0 a
V4	T0	0.0 ± 0.0 b	18.3 ± 2.9 d	100.0 ± 0.0 a
	T1	25.0 ± 3.0 a	83.3 ± 17.6 ab	100.0 ± 0.0 a
	T2	0.0 ± 0.0 b	33.3 ± 5.8 cd	100.0 ± 0.0 a
	T3	0.0 ± 0.0 b	25.0 ± 6.2 d	100.0 ± 0.0 a
P(>F) V		<0.001***	<0.001***	ns
P(>F) T		<0.001***	<0.001***	ns
P(>F) V*T		<0.001***	<0.001***	ns

Numbers in the same column followed by the same letter are not significantly different at a 5% degree of freedom threshold. WAS: weeks after sowing; T0: non-inoculated varieties; T1: varieties inoculated with nematodes + Fusarium; T2: varieties inoculated with nematodes + Fusarium then treated with aqueous neem extract; T3: varieties inoculated with nematodes + Fusarium then treated with synthetic pesticides. (V1: White of the Center (togologo); V2: White variety of Adamaoua (lambadidi); V3: Improved Yellow variety (IRAD 1112); V4: Yellow variety of the Center (Bété))

3.6. Effects of Treatments and Variety on the Severity of *Fusarium* Wilt in Sweet Potato

The evolution of the degree of infection by the disease (Severity) in the different plots shows variation over time and according to varieties. These results indicate a general increase in the level of severity in the plots across different varieties over time (Table 5). The effects of variety, treatment, and the variety-treatment interaction are significant ($P < 0.05$). Significant differences were observed ($P < 0.05$) for each observation period (5, 7, and 9 SAS). Varieties V2 and V3 exhibited the lowest severities (8.67 ± 1.15; 18.33 ± 7.64; 11.67 ±2.89; 8.33 ±1.53; 18.33 ±4.16; 28.33 ±4.73; 13.33 ± 3.06 and 15.00 ± 5.00, respectively for T0, T1, T2, and T3). Treatment with neem extract (T2) reduced severity rates by 60 to 80% in the different plots compared to the control (T0), similar to the chemical fungicide (T3).

Table 5. *Effect of Treatments on the Severity of Fusarium Wilt in Sweet Potato.*

Varieties	Treatments	5 SAS	7 SAS	9 SAS
V1	T0	3.33 ± 1.44 cd	15.00 ± 5.00 b	23.33 ± 2.89 bc
	T1	13.33 ± 2.89 a	31.67 ± 7.64 a	63.33 ± 5.77 a
	T2	6.67 ± 1.53 b	11.67 ± 2.89 bc	25.00 ± 5.00 bc
	T3	3.33 ± 1.53 cd	11.67 ± 2.08 bc	23.33 ± 5.77 bc
V2	T0	0.00 ± 0.00 e	0.00 ± 0.00 f	8.67 ± 1.15 e
	T1	0.00 ± 0.00 e	8.33 ± 1.53 cde	18.33 ± 7.64 cd
	T2	1.67 ± 0.29 de	1.67 ± 1.15 f	11.67 ± 2.89 de
	T3	3.33 ± 1.26 cd	3.33 ± 1.53 ef	8.33 ± 1.53 e
V3	T0	3.33 ± 1.04 cd	5.00 ± 2.00 def	18.33 ± 4.16 cd
	T1	5.00 ± 1.00 bc	13.33 ± 4.16 bc	28.33 ± 4.73 b
	T2	6.67 ± 2.08 b	8.33 ± 2.08 cde	13.33 ± 3.06 de
	T3	1.67 ± 0.58 de	5.00 ± 1.00 def	15.00 ± 5.00 de
V4	T0	5.00 ± 1.00 bc	5.00 ± 1.50 def	13.33 ± 3.51 de
	T1	6.67 ± 1.15 b	15.00 ± 4.58 b	28.33 ± 2.89 b
	T2	1.67 ± 0.29 de	5.00 ± 2.00 def	10.00 ± 2.00 e
	T3	5.00 ± 2.00 bc	10.00 ± 1.00 bcd	18.33 ± 5.77 cd
P(>F) V		<0.001***	<0.001***	<0.001***
P(>F) T		<0.001***	<0.001***	<0.001***
P(>F) V*T		<0.001***	0.002825 **	<0.001***

Numbers in the same column followed by the same letter are not significantly different at a 5% degree of freedom threshold. WAS: weeks after sowing; T0: non-inoculated varieties; T1: varieties inoculated with nematodes + Fusarium; T2: varieties inoculated with nematodes + Fusarium then treated with aqueous neem extract; T3: varieties inoculated with nematodes + Fusarium then treated with synthetic pesticides. (V1: White of the Center (togologo); V2: White variety of Adamaoua (lambadidi); V3: Improved Yellow variety (IRAD 1112); V4: Yellow variety of the Center (Bété))

Effect of Treatments and Variety on Sweet Potato Yield

The yields in weight of marketable tubers (RC) in tons per hectare and the yield in the number of tubers per plant are determined to evaluate the effect of extracts and varieties on yield gains compared to other treatments (Table 6). The variety x treatment interaction, the effect of variety, and the effect of treatment are all significant ($P < 0.001$) for the four tested varieties. The highest yields are obtained with the treatment using aqueous neem seed extract (T2) and the chemical fungicide (T3) across all varieties.

Table 6. Effect of Treatments and Variety on Sweet Potato Yield.

Varieties	Treatments	Number of Tubers / Plant	Yield (t/ha)
V1	T0	2.34 ± 0.88 d	1.74 ± 0.62 f
	T1	1.96 ± 0.58 d	2.25 ± 1.01 f
	T2	2.75 ± 0.76 cd	4.10 ± 0.68 e
	T3	4.06 ± 1.26 abc	4.91 ± 0.77 de
V2	T0	2.13 ± 0.52 d	3.73 ± 0.73 e
	T1	2.25 ± 0.57 d	2.12 ± 0.53 f
	T2	4.62 ± 0.99 a	7.64 ± 0.93 a
	T3	4.12 ± 0.78 abc	7.52 ± 1.36 ab
V3	T0	1.95 ± 0.35 d	1.84 ± 0.38 f
	T1	1.67 ± 0.69 d	1.25 ± 0.31 f
	T2	2.85 ± 1.76 bcd	5.81 ± 0.64 cd
	T3	4.22 ± 0.78 ab	6.18 ± 0.94 c
V4	T0	2.26 ± 0.02 d	1.94 ± 0.57 f
	T1	2.00 ± 0.33 d	1.47 ± 0.41 f
	T2	4.31 ± 1.06 a	5.70 ± 0.70 cd
	T3	4.08 ± 0.69 abc	6.34 ± 0.28 bc
P(>F) V		0.2434 ns	<0.001***
P(>F) T		<0.001***	<0.001***
P(>F) V*T		0.4885 ns	0.01893 *

Numbers in the same column followed by the same letter are not significantly different at a 5% degree of freedom threshold. WAS: weeks after sowing; T0: non-inoculated varieties; T1: varieties inoculated with nematodes + Fusarium; T2: varieties inoculated with nematodes + Fusarium then treated with aqueous neem extract; T3: varieties inoculated with nematodes + Fusarium then treated with synthetic pesticides. (V1: White of the Center (togologo); V2: White variety of Adamaoua (lambadidi); V3: Improved Yellow variety (IRAD 1112); V4: Yellow variety of the Center (Bété))

3.7. Total Phenolic Compound Content in the Leaf Tissues of Sweet Potato Varieties

The phenolic compound content is highest in the leaves of variety V1 inoculated with *Fusarium* + nematode and treated with aqueous neem extract (T2: 1103.90 ± 70.75 mg/g DM), followed by plants inoculated with *Fusarium* + nematode and treated with synthetic pesticides (T3: 1085.57 ± 58.20 mg/g DM) of the same variety, and variety V3 inoculated with *Fusarium* + nematode and treated with synthetic pesticides (1003.57 ± 63.09 mg/g DM). In contrast, lower contents were recorded for variety V2 inoculated with *Fusarium* + nematode (230.96 ± 52.04 mg/g DM), V1 inoculated with *Fusarium* + nematode and treated with aqueous neem extract (T2: 234.27 ± 69.48 mg/g DM), and variety V3 that received no treatment (230.98 ± 72.15 mg/g DM). A significant difference ($P < 0.05$) is recorded for the variety x treatment interaction (Figure 2).

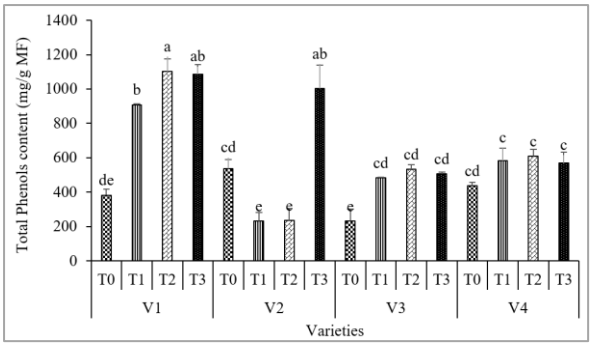

Figure 2. Variation of Phenolic Compound Content in the Leaves of Sweet Potato Varieties T0: non-inoculated varieties; T1: varieties inoculated with nematodes + Fusarium; T2: varieties inoculated with nematodes + Fusarium then treated with aqueous neem extract; T3: varieties inoculated with nematodes + Fusarium then treated with synthetic pesticides. V1: White variety of the Center (togologo); V2: White variety of Adamaoua (lambadidi); V3: Improved Yellow variety (IRAD 1112); V4: Yellow variety of the Center (Bété).

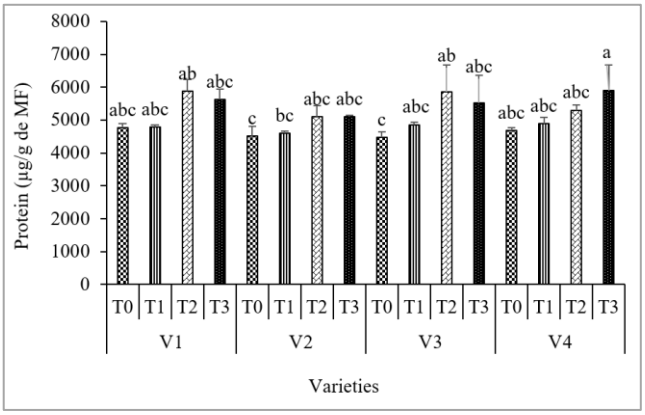

Figure 3. *Variation of Total Soluble Protein Content in the Leaves of Sweet Potato Varieties. T0: non-inoculated varieties; T1: varieties inoculated with nematodes + Fusarium; T2: varieties inoculated with nematodes + Fusarium then treated with aqueous neem extract; T3: varieties inoculated with nematodes + Fusarium then treated with synthetic pesticides. V1: White variety of the Center (togologo); V2: White variety of Adamaoua (lambadidi); V3: Improved Yellow variety (IRAD 1112); V4: Yellow variety of the Center (Bété).*

3.8. Total Protein Content in the Leaf Tissues of Sweet Potato Varieties

A significant difference ($P < 0.05$) is recorded for the variety x treatment interaction for the total protein content measured (Figure 3). In terms of proteins, variety V4 inoculated with *Fusarium* + nematode and treated with synthetic pesticides has the highest value (T3: 5894.84 ± 348.64 µg/g DM), followed by variety V1 inoculated with *Fusarium* + nematode and treated with aqueous neem extract (T2: 5869.46 ± 367.65 µg/g DM) and variety V3 inoculated with *Fusarium* + nematode and treated with aqueous neem extract (T2: 5866.47 ± 302.19 µg/g DM). In contrast, variety V2 (T0: 4513.97 ± 305.48 µg/g DM) and variety V3 (T0: 4473.38 ± 168.97 µg/g DM) that received no treatment have the lowest protein contents.

3.9. Variation of Oxidative Enzyme Activity in the Leaf Tissues of Sweet Potato Varieties

3.9.1. Peroxidase Activity

A significant difference is observed ($P < 0.05$) for the variety x treatment interaction for the peroxidase parameter evaluated (Figure 4). The highest peroxidase (POX) activity was produced by variety V1 inoculated with *Fusarium* + nematode and treated with aqueous neem extract (T2: 2.84 ± 0.11 ΔA420/min/mg DM), followed by variety V1 inoculated with *Fusarium* + nematode and treated with aqueous neem extract (T2: 1.94 ± 0.04 ΔA420/min/mg DM) and V4 inoculated with *Fusarium* + nematode and treated with synthetic pesticides (T3: 1.84 ± 0.06 ΔA420/min/mg DM). The lowest POX content was produced by variety V2 inoculated with *Fusarium* + nematode (T1: 0.67 ± 0.05 ΔA420/min/mg DM) and variety V3 that received no treatment (T0: 0.67 ± 0.05 ΔA420/min/mg DM).

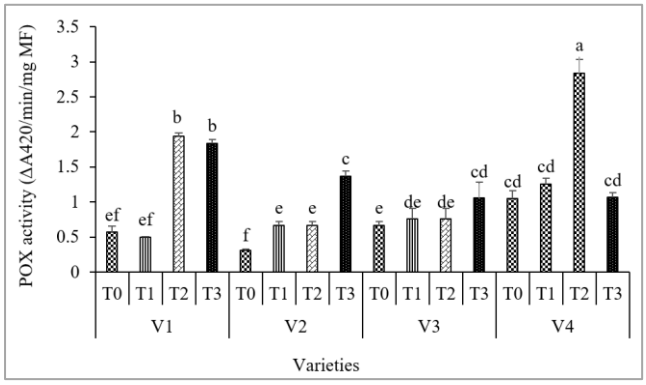

Figure 4. *Variation of Peroxidase Content in the Leaves of Sweet Potato Varieties. T0: non-inoculated varieties; T1: varieties inoculated with nematodes + Fusarium; T2: varieties inoculated with nematodes + Fusarium then treated with aqueous neem extract; T3: varieties inoculated with nematodes + Fusarium then treated with synthetic pesticides. V1: White variety of the Center (togologo); V2: White variety of Adamaoua (lambadidi); V3: Improved Yellow variety (IRAD 1112); V4: Yellow variety of the Center (Bété).*

3.9.2. Phenylalanine Ammonia-Lyase Activity

A significant difference ($P < 0.05$) is recorded for the variety x treatment interaction (Figure 5). The phenylalanine ammonia-lyase (PAL) activity observed in the leaves of sweet potato variety V1 inoculated with *Fusarium* + nematode and treated with aqueous neem extract (T2: 60.67 ± 6.11 ΔA290/min/mg DM) and inoculated with *Fusarium* + nematode and treated with synthetic pesticides (T3: 62.17 ± 3.62 ΔA290/min/mg DM) is the highest. The lowest activity is observed in variety V1 (T0: 13.13 ± 1.60 ΔA290/min/mg DM) and V2 (T0: 14.72 ± 1.33 ΔA290/min/mg DM) that received no treatment.

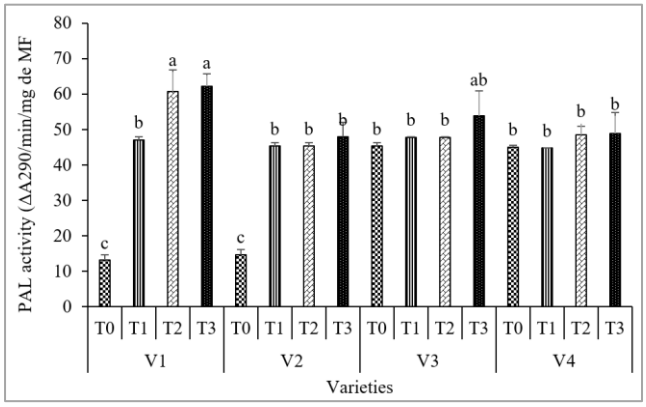

Figure 5. *Variation of Phenylalanine Ammonia-Lyase Content in the Leaves of Sweet Potato Varieties. T0: non-inoculated varieties; T1: varieties inoculated with nematodes + Fusarium; T2: varieties inoculated with nematodes + Fusarium then treated with aqueous neem extract; T3: varieties inoculated with nematodes + Fusarium then treated with synthetic pesticides. V1: White variety of the Center (togologo); V2: White variety of Adamaoua (lambadidi); V3: Improved Yellow variety (IRAD 1112); V4: Yellow variety of the Center (Bété).*

4. Discussion

The results obtained in the greenhouse regarding the effect of different applications of plant protection products on the growth parameters of sweet potato show that the number of branches was not affected by the treatments applied or by the varieties of sweet potato studied, throughout the observation periods. This result might be attributed to the genetic stability of this species, which is hexaploid and reproduces exclusively through vegetative means [40]. However, the effect of variety is significant during the 7 and 9 WAP periods. The variability observed in the vegetative development of the plants might result from genetic variation among the sweet potato genotypes tested [41]. Furthermore, Ngatsi *et al.* [42] reported that the use of yellow laurel extracts against root rot in cassava, do not effect on the plant growth. The observation of the evolution of infestation rates of the plots by the disease shows variation over time and according to the varieties. Analysis of variance reveals significant differences between the incidence values obtained in the plots, except in the last week of observation where all plots show an incidence of 100%. Treatment with neem extract reduced severity rates by 60 to 80% in the different plots compared to T1, similar to the chemical fungicide (T3). This action may be due to the presence of sesquiterpenes, flavonoids, and calcium carbonate in the extract, which are known for their antifungal properties [22]. The yields in weight of marketable tubers (RC) expressed in tons per hectare and the yield in the number of tubers per plant were determined to evaluate the effect of extracts and varieties on yield gains compared to other treatments. The variety x treatment interaction, the effect of variety, and the effect of treatment are all significant for the four tested varieties. The highest yields were obtained with the treatment using aqueous neem seed extract and the chemical fungicide across all varieties. The height of sweet potato plants is significantly affected by the treatments applied and the variety. However, the variety-treatment interaction is not significant). At 9 WAP, the treatment effect and the variety effect are significant. These results could be explained by the fact that raw neem extract contains, in addition to its main active ingredient, azadirachtin, other metabolites capable of indirectly inducing plant growth. Furthermore, while evaluating the antifungal effect of neem aqueous extract on the development of *Phakopsora pachyrhizi*, Ndogho *et al.* [43] observed an increase in vegetative growth, particularly in terms of plant height and the number of leaves on treated soybean plants. The phenolic compound content was significantly higher in the sweet potato leaves from the treatments than in the control. This could be explained by the presence of the disease and nematodes, as well as by the treatments applied. Indeed, phenolic compounds are a very important group of secondary metabolites involved in plant resistance to pathogens. According to Lawrence *et al.* [12], phenolic compounds are present in plant organs, conferring antimicrobial properties. Moreover, the protein content in sweet potato plants inoculated with *Fusarium* + nematode and treated with synthetic pesticides, as well as those inoculated and treated with aqueous neem extract, is relatively higher than in the control sweet potato plants. This may be attributed, on one hand, to the infection by the pathogen (*Fusarium*) and nematodes, and on the other hand, to the treatments that stimulate the plant defense mechanisms, allowing them to resist the aggressions caused by parasites. Numerous studies indicate that protein synthesis is coded by genes and can be expressed more markedly when the plant undergoes stress, such as infection by pathogens or elicitors [44, 45]. Singh *et al.* [46] also confirmed that the production of defense proteins is an important mechanism of resistance in plants against pathogens. This reinforces the idea that treatments with neem extract and other plant protection products could stimulate protein production, thereby contributing to the plants' defense against *Fusarium* and other pathogens. PR (Pathogenesis-Related) proteins can inhibit the growth of pathogens and/or the germination of spores. They also act as antimicrobial agents, hydrolases, and proteinase inhibitors [47, 48]. Arya and Tiagi [49] demonstrated a significant accumulation of these proteins in galls formed by *Meloidogyne incognita* on carrot (Daucus carota), particularly in the perigall tissue [50]. Regarding the activity of oxidative enzymes, it was observed that phenylalanine ammonia-lyase (PAL) is elevated in the leaves of V1. PAL enhances the production of cinnamic acid, thus playing a role in necrosis formation processes [48]. Giebel [51] was the first to study changes in PAL activity in hypersensitivity reactions induced by bioaggressors. His work demonstrated that, in susceptible potato plants, PAL activity decreased with the age of the plants, but remained higher in diseased plants. A similar phenomenon was observed in resistant plants, where an increase in PAL activity was noticeable only early in the infection, with activity becoming higher later on. The enzymatic activity of peroxidase (POX) is higher in sweet potato plants inoculated with *Fusarium* + nematode and treated with aqueous neem extract than in control plants. According to de Ascensao and Dubery [52], POX is linked to the host plant's defense response and plays a crucial role in strengthening the plant's cell wall, thereby limiting the spread of the pathogen. Passardi *et al.* [53] showed that the production of POX can prevent chemical and biological attacks by reinforcing physical barriers or generating a strong production of free radicals to counterattack microorganisms.

5. Conclusion

From this study, which aimed to test the tolerance of four sweet potato varieties treated with aqueous neem seed extract against nematodes and *Fusarium* wilt, it emerges that treatment with aqueous neem seed extract reduced severity rates by 60 to 80% in the different plots compared to the control (T1), with an effectiveness comparable to that of the chemical fungicide (T3). The highest yields were obtained with the treatment using aqueous neem seed extract and the chemical fungicide across all varieties. Among these, the White variety

of Adamawa (V2) and the IRAD 1112 variety (V3) exhibited exhibited the highest yields. Additionally, sweet potato plants inoculated with *Fusarium* + nematode showed higher contents of phenolic compounds, proteins, and increased enzymatic activity of peroxidase (POX). further characterization of sweet potato nematodes in Cameroon and testing of other biocontrol methods are required.

Abbreviations

PDA	Potato Dextrose Agar
PAL	Phenylalanine Ammonia-Lyase
POX	Peroxidase
WAP	Week After Planting

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Author Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

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