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Comparative Efficacy of Two Bionematicides (Neem/Chili-Based and Trichoderma harzianum-Based) Against Tomato Parasitic Nematodes and Their Impact on Yield in Bobo-Dioulasso, Burkina Faso

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Abstract

This study assessed the biological efficacy of two bionematicides—NEMATISAIN (a blend of 6% neem oil, 2% chili pepper extract, 1% citrus terpene extract, and trace elements) and FERTISAIN (containing 3% Gamma lactone from Trichoderma harzianum extracts, 1% citrus terpene oil, 0.5% clove oil, and essential minerals)—in comparison to the chemical nematicide VELUM (Fluopyram 400g/L). The trials were conducted at the Léguéma market gardening site in Bobo-Dioulasso, western Burkina Faso, known for its high nematode infestation. All evaluated products demonstrated significant effectiveness in controlling parasitic nematodes by markedly reducing the population densities of major species, including Meloidogyne javanica, Pratylenchus brachyurus, Scutellonema cavenessi, and Helicotylenchus dihystera.Crucially, the nematicide treatments led to substantial increases in tomato yield compared to the untreated control.1 The bionematicides, particularly FERTISAIN (52.76%), surpassed the yield gains of the chemical standard VELUM (37.79%), with NEMATISAIN also showing strong performance (35.48%). These results confirm that both plant-extract-based and microbial-based bionematicides offer viable, effective, and environmentally preferable alternatives to chemical control for managing tomato parasitic nematodes and boosting productivity in the region.

Keywords: bionematicide, parasitic nematodes, tomato, yield gain, Meloidogyne javanica, Trichoderma harzianum, Burkina Faso.

INTRODUCTION

In Burkina Faso, tomatoes are in second place after onions in terms of cultivated areas and production volume [1]. Tomatoes are in second place after onions with an estimated production of more than 200,519 tons [2]. It remains the most profitable vegetable crop in the country. Indeed, an assessment of the gross margins generated by the crops shows that that of tomatoes amounted to about 5.5 billion CFA francs, followed by onions with 4.5 billion CFA francs [2]. However, in recent years, there has been a decline in yields from 12.5 tons/ha in 2012 to 10 tons/ha in 2017 [3]. It is estimated that the damage caused by RKN globally results in an annual economic loss of more than US$100 billion, or about 12.6% of total crop losses [4]. These yield declines are mainly due to a complex of pests and diseases [5]. To deal with these pests, chemical control using synthetic pesticides is the most commonly used by producers [5]. Among the pests, root-knot nematodes of the genus *Meloidogyne* are known to be very destructive to tomatoes grown in greenhouses and in open fields around the world and cause yield losses of between 30 and 40% [6]. To deal with these pests, the use of synthetic chemical pesticides is the most widely used solution. In this context of misuse of synthetic chemical pesticides with serious consequences by market gardeners, it is necessary to seek to develop other, less damaging strategies to control pests and diseases while reducing the harmful effects on human and animal health and/or the environment. It is in this perspective that the present study aims to evaluate the effectiveness of two (2) nematicides NEMATISAIN and FFERTISAIN in comparison with a chemical nematicide VELUM in the fight against tomato parasitic nematodes in Burkina Faso.

METHODOLOGY

Study site

The experiment was carried out on the market gardening perimeter of Léguéma. It is a village located 16 km from the city of Bobo-Dioulasso, at the north-east exit. Its surface area is estimated at 4800 ha with 4200 producers. It is a reference site in the field of nematological research because it is known to be very infested by nematodes parasitic on vegetable crops, mainly of the genus *Meloidogyne*. The soil is sandy and suitable for the development of the root-knot nematodes *Meloidogyne* spp. The site's geographical coordinates are 11°14′2.70" North latitude, 4°09′24.10" West longitude and an elevation of 323 meters. The climate of the area is of the South Sudanese type [7] and average temperatures vary between 24.9°C and 30.2°C with a relatively low temperature range of 5.3°C. Like the commune of Bobo-Dioulasso, the average annual rainfall in Léguéma has fluctuated between 748.3 and 1,375.5 mm over the last ten years [8]. The nematode extraction work was carried out at the Nematology Laboratory of the Institute of the Environment and Agricultural Research (INERA)/Farako-Bâ station located in Bobo-Dioulasso.

Material Biological and chemical nematicides used

Two bio-nematicides and one chemical nematicide were used. They are: - the bio-nematicide NEMATISAIN based on neem oil (6%), chili extract (2%), citrus terpenic extract (1%) and trace elements. -the Bio-nematicide FERTISAIN a root fertilizer based on *Trichoderma harzianum* (3% Gamma lactone from *T. harzianum* extracts) containing additionally 1% citrus terpenic oil, 0.5% clove oil, 2% Magnesium Oxide, 0.1% manganese and 0.1% Zinc. - the chemical nematicide or reference control namely VELUM PRIME SC (Fluopyram 400g/L).

Methods Experimental design

The experimental design used is a complete randomized block comprising 4 treatments and 4 replicates. The surface area of the elementary plot is 28m² (7m x 4m). Plants should be transplanted at 80 cm spacing between rows and 50 cm in the row. The distance between two consecutive elementary plots is 1 m. The different treatments are recorded in Table 1.

Table 1: Quantity of products per treatment

Treatment	Quantity /ha	Quantity /28m²
T 1: Untreated	-	-
T 2: VELUM	1.25	3.5 ml/28m²
T 3: NEMATISAIN	5 l/ha	14 ml/28m²
T 4: FERTISAIN	3 l/ha	8.4 ml/28m²

Nursery production and transplanting of tomato plants

The tomato seeds of the Cobra variety were sown in trays containing sterilized soil. Sowing was done in a continuous line spaced 20 cm apart between the rows. The sowing depth was about 1 cm. After 28 days of growth, the seedlings were transplanted into the field. 28-day-old tomato plants were transplanted to each elementary plot (6 rows per elementary plot and 15 plants on average per row). Plants were transplanted at spacings of 80 cm between the rows and 50 cm on the row.

Method of application of processing

The trial plot is ploughed, harrowed and levelled. The product is applied to the base of each tomato plant on wellmoist soil using the following process:

- For NEMATISAIN, 14 ml of the product is put in a sprayer containing 10 liters of water and applied in an open line at the foot of the plants. A second application is made 15 days after the first treatment;
- For FERTISAIN, 8.4 ml of the product is put in a sprayer containing 08 liters of water and applied in an open furrow at the foot of the plants. A second application is made 15 days after the first treatment;
- For VELUM, 3.5 ml of the product is put in a sprayer containing 05 liters applied in an open furrow at the foot of the plants.

Fertilization and maintenance

Mineral fertilizers consisting of NPK (15-15-15) at a rate of 300 kg/ha and urea (46%) at a rate of 150 kg/ha were applied according to the periods recommended by the research (15 and 45 days after transplanting). Manual weeding was carried out as needed. A weeding was carried out at the beginning of the fruiting of the plants with the installation of the stakes.

Observed and measured parameters - Nematological observations

Nematological observations concerned the two penultimate lines of each row of the elementary plot. Nematodes are extracted from soil and roots using the methods of [9], [10]. Population densities are expressed in number of nematodes/dm3 of soil and number of nematodes/g of roots. Nematological observations concerned the population levels of parasitic nematodes in the soil at tomato transplanting, on the 30th day after transplanting (JAR), on the 60th day and on the 90th day (at harvest). Nematodes extracted from the roots were assessed at the 30th JAR, 60th JAR, and 90th JAR. The gall-count was measured at the time of tomato harvest according to the Indexing Table for Root-knot Nematodes (From [11]).

Agronomic observations

Agronomic observations were made on ten (10) tomato plants identified at transplanting and concerned the height of the plants from the collar to the last leaf on the 45th day (days after transplanting). The number of flowers/plant was also counted at the 45th JAR. Tomato yield was estimated on the two (2) central lines during 3 successive harvests. The harvest yield was measured on the two central lines of each elementary plot, was calculated from the harvests carried out on the two central lines of each elementary plot and extrapolated to the hectare.

Statistical data processing and analysis

The Excel 2016 spreadsheet was used for data entry and graphing. The statistical software XLSTAT 2016 was used for the analysis of variance (ANOVA) and the Newman-Keuls test for the separation of means. This test performs all paired comparisons of means, using the distribution of Student ranges. Due to high coefficients of variation, the nematological data have previously undergone a logarithmic transformation (Log10 (X +1) before ANOVA.

RESULTS AND DISCUSSION

Results Stands of parasitic nematodes associated with tomatoes

The populations of plant-parasitic nematodes observed in the soil at the Léguéma site during tomato transplanting are given in Table 2. They are represented by *Meloidogyne javanica*, *Pratylenchus brachyurus*, *Scutellonema cavenessi*, *Helicotylenchus dihystera*, *Tylenchorhynchus sp.*, *Xiphinema sp.* and *Criconemella onoensis*. Four (4) nematodes observed in 100% of the samples at high average population densities (between 588 and 6943 nematodes/dm^3 of soil) can be considered as the most damaging to tomatoes on the market gardening perimeter of Léguéma. These nematodes are represented *Meloidogyne javanica*, *Pratylenchus brachyurus*, *Scutellonema cavenessi* and *Helicotylenchus dihystera*.

Table 2: Frequency and abundance of tomato-parasitic nematode stands observed in tomato transplanting

Nematode densities Frequency	**(number de nematodes/dm^3 de soil) (%)**	**Minimum**	**Maximum**	**Average**	**Standard deviation**
Meloidogyne javanica	100.00	60	1320	624	416
Pratylenchus brachyurus	100.00	20	3560	588	900
Scutellonema cavenessi	100.00	160	4480	1873	1414
Helicotylenchus dihystera	100.00	1320	15540	6943	3954
Tylenchorhynchus sp.	56.25	0	260	50	74
Criconemella onoensis	37.50	0	40	9	13
Xiphinema sp.	25.00	0	40	8	14
Total	-	2720	21280	10093	5606

Effect of treatments on tomato parasitic nematodes

The effect of bionematicides compared to a chemical nematicide concerned the main plant-parasitic nematodes of tomatoes represented by *Meloidogyne javanica*, *Pratylenchus brachyurus*, *Scutellonema cavenessi* and *Helicotylenchus dihystera*. The dynamics of plant parasitic nematode communities as a function of treatments are given in Figures 2 and 3. The root-knot nematode *Meloidogyne javanica* was observed in soil samples (Figure 1A) and in tomato roots (Figure 1B). The analysis of variance (ANOVA) did not show a significant difference between the treatments before tomato transplanting with population densities between 500 and 825 nematodes/dm3 of soil ($P > 0.05$). Nematological observations made on day 30 after transplanting (JAR) showed an efficacy of the neembased bionematicide (NEMATISAIN) and the chemical nematicide VELUM (Fluopyram 400g/l) compared to the untreated control and the FERTISAIN treatment (based on *Trichoderma harzianum*) ($P < 0.05$). Observations made at the 60th and 90th JAR (harvest) showed an efficacy of nematicide treatments compared to the Control ($P < 0.06$). Bionematicides (NEMATISAIN and FERTISAIN) and chemical nematicide

(VELUM) had a significant effect on *Meloidogyne javanica* populations extracted from tomato roots (P < 0.05). At harvest, population densities were 1119 nematodes/g of root on the Control and 84 nematodes/g of roots on the T4 treatment (FERTISAIN based on *T. harzianum*).

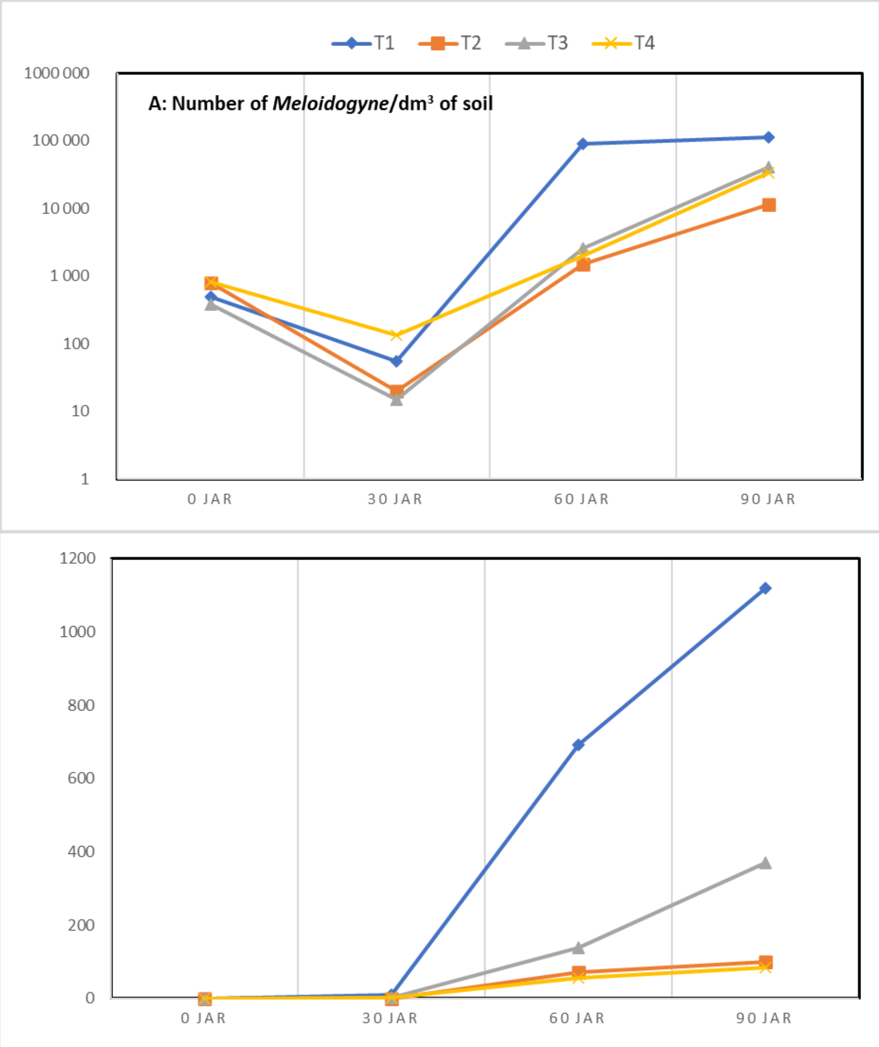

Figure 1: Effect of nematicide treatments on population densities of Meloidogyne javanica A) in soil samples and B) in roots in soil samples during the tomato development cycle.
T1: Untreated control; T2: VELUM (Fluopyram, 400g/L) at 1.25L/ha; T3: NEMATISAIN (neem oil (6%), chili pepper extract (2%), citrus terpene extract (1%) and trace elements) at a dose of 5L/ha; T4: FERTISAIN (3% Gamma lactone from T. harzianum extracts) at a dose of 3L/ha.

Changes in the population densities of nematodes of the nematodes of the nematodes *Pratylenchus brachyurus*, *Scutellonema cavenessi* and *Helicotylenchus dihystera* observed only in soil are shown in Figure 2 below. The populations of nematodes extracted from the roots were very low or even non-existent. For tomato transplanting, the population densities of *Pratylenchus brachyurus* (Figure 2A) are comparable for all treatments with levels between 140 and 300 nematodes/dm3 of soil (P > 0.05). From the 30th to the 90th JAR, population densities remained high for the Control with levels above 1000 nematodes/dm3 of soil compared to densities between 40 and 315 nematodes/dm3 of soil observed on the other treatments (P < 0.05). The population densities of S. cavenessi and H. dihystera (Figure 2B and 2C) did not show a difference between the different tomato transplanting treatments (P > 0.05). The efficacy of the bionematicides (NEMATISAIN and FERTISAIN) and the chemical nematicide (VELUM) was not observed on these 2 nematodes at the 30th JAR (P > 0.05). At the 60th JAR, the population densities of S. cavenessi are high on the Control (3535 nematodes/dm3) and low bionematicide and chemical treatments (levels between 190 and 325 nematodes/dm3 of soil) (P < 0.05). At the 60th JAR, the Control was very infested with H. dihystera with 12,345 nematodes/dm3 of soil compared to averages of between 960 and 1505 nematodes/dm3 of soil for the other treatments (P < 0.05). At the 90th JAR, the VELUM treatment allowed a reduction in the populations of S. cavenessi with 75 nematodes/dm3 of soil, although higher densities on the Control and the bionematicide treatments (P < 0.05). The different treatments did not show a significant difference for H. dihystera at the 90th JAR (P > 0.05).

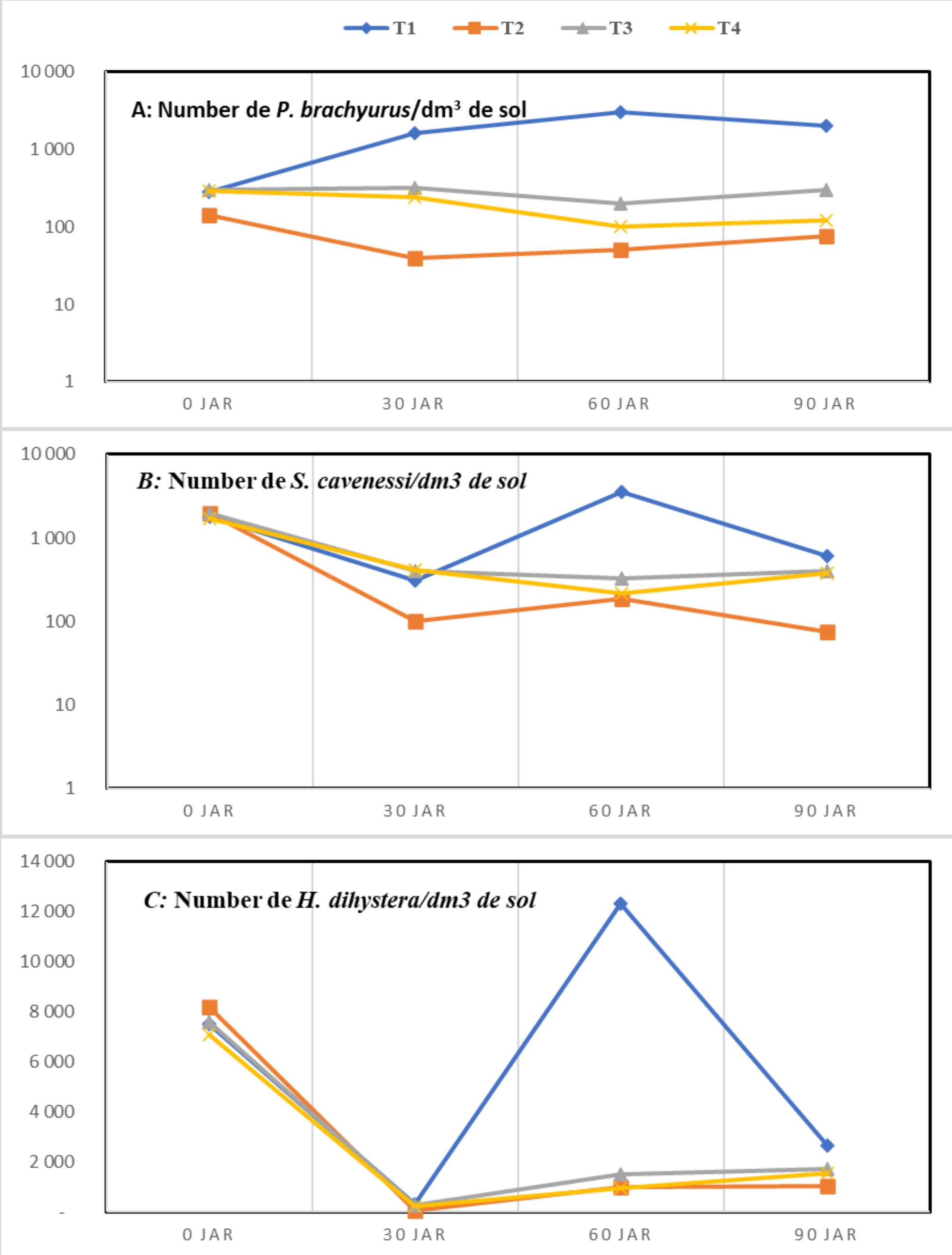

Figure 2: Effect of nematicide treatments on population densities of other plant-parasitic nematodes (Pratylenchus brachyurus, Scutellonema cavenessi, Helicotylenchus dihystera) in soil samples during the tomato development cycle
T1: Untreated control (TNT); T2: VELUM (Fluopyram, 400g/L) at 1.25L/ha; T3: NEMATISAIN (neem oil (6%), chili pepper extract (2%), citrus terpene extract (1%) and trace elements) at a dose of 5L/ha; T4: FERTISAIN (3% Gamma lactone from T. harzianum extracts) at a dose of 3L/ha

Effect of different treatments on yield components and tomato yield Effect of different treatments on tomato yield components

Plant height and number of flowers were measured on the 45th JAR and the data reported in Table 3. Nematicides did not affect plant height ($P > 0.05$). On the other hand, the number of flowers per plant was lower on the Control compared to the T2 treatment (VELUM) ($P < 0.05$). The weight of fresh tomato roots measured at harvest was higher on the bionematicides FERTISAIN (T4 based on *Trichoderma harzianum*) and NEMATISAIN (T3 based on neem, chili, citrus and trace elements) with 22.5g and 17.2g respectively compared to the Control (11.8g) (P <

0.01). The gall index was measured at harvest from fresh tomato roots according to the Indexation Table for Rootknot Nematodes (from [11]) (Table 3). Low indices were observed on T2 (VELUM chemical nematicide) and T4 (FERTISON bionematicide) with scores of 1.8 and 2.1 respectively compared to Control (6.1) and T3 (NEMATISAIN) (2.1) ($P < 0.001$).

Table 3: Effect of nematicide treatments on tomato yield components

Treatments	Yield			
	Height(cm)	**Flowers (number)**	**Weight of the roots (g)**	**Galls index**
T1 : Untreated control	18.5 A	30.9 A	11.8 A	6.1 C
T2 : VELUM (1.25L/ha)	21.4 A	46.6 B	13.9 AB	1.8 A
T3 : NEMATISAIN (5 L/ha)	20.8 A	41.3 AB	17.2 B	4.1 B
T4 : FERTISAIN (3 L/ha)	20.1 A	40.0 AB	22.5 C	2.1 A
Probability (P)	> 0.05	< 0.05	< 0.01	< 0.001
Signification	ns	*	**	***

*The means followed by the same letter in a column are not significantly different according to the Newman-Keuls test at the thresholds of: *: 5%; **: 0,01; ***: 0,001. NS: Not significant.*

Effect of different treatments on yield

The treatment based on chemical nematicide (T2: VELUM at 1.25 L/ha) with 14.24 T/ha, the bionematicides (T3: NEMATISAIN and T4: FERTISAIN) with 14.00 and 15.79 T/ha respectively improved the yield of the tomato compared to the untreated control with 10.33 T/ha ($P < 0.01$). Yield gains of more than 35% were obtained on the nematicide-treated plots compared to the untreated control (Table 4).

Table 4: Effect of treatments on tomato yield

Treatments	***Fruit yield/ha***	***Yield gain (%)***
T1 : Untreated control	10.33 B	
T2 : VELUM	14.24 A	37.79
T3 : NEMATISAIN (5 L/ha)	14.00 A	35.48
T4 : FERTISAIN (3 L/ha)	15.79 A	52.76
Probability (P)	< 0.01	-
Signification	**	-

*Means followed by the same letter in a column are not significantly different according to the Newman-Keuls test at the thresholds of: **: 0.01*

Discussion Stands of parasitic nematodes associated with tomatoes

The analysis of soil samples from tomato transplanting has identified 7 genera of nematode parasites associated with tomatoes on the market garden site of Léguéma. Our study showed that 4 main genera of nematodes are present in the soil, namely *Meloidogyne, Pratylenchus*, *Scutellonema* and *Helicotylenchus*. Root-knot nematodes are considered to cause the most damage to tomatoes and the chemical nematicides developed have always been directed towards this major tomato pest. Several studies conducted in Burkina Faso have shown that the main parasitic nematodes associated with tomatoes are represented by *Helicotylenchus* sp., *Scutellonema* sp., *Pratylenchus* sp. and *Meloidogyne* spp. ([12]; [13]). In Ghana, [14] have identified 9 species of tomato-associated parasitic nematodes, the most important of which are *Helicotylenchus* spp., *Hoplolaimus* spp., *Meloidogyne* spp., *Pratylenchus* spp., *Rotylenchulus* spp., *Scutellonema* spp. Several species of *Pratylenchus* spp. have been identified as tomato root parasitic nematodes in several countries ([15]; [16]).

Efficacy of bionematicides and chemical nematicide on tomato parasitic nematode stands

The NEMATISAIN bionematicide (based on neem oil (6%), chili pepper extract (2%), citrus terpene extract (1%) and trace elements) has shown its effectiveness against tomato parasitic nematodes. Our results are in agreement with the work of [17] in Benin which showed that the application of neem cakes in tomato plots led to a reduction in populations of root-knot nematodes of the genus *Meloidogyne* in the soil and in the roots. Several previous studies have also reported that the nematicide effect of neem derivatives against root-knot nematodes is due in particular to azadirachtin and other triterpenoids ([18]; [19]). Other authors have obtained low gall index on tomato plants treated with neem almond powder, reflecting the inhibitory effect of neem on the development of root-knot nematodes ([20]; [21]). The effectiveness of clove (*Syzygium aromaticum*) could be explained by eugenol, an active ingredient contained in clove oil, which has been proven by [22] to act as a potent nematicide. [23] who studied the nematicide activity of cloves against the root-knot nematode *Meloidogyne incognita* in greenhouses, found that cloves were effective at killing nematodes with an EC_{50} value (effective concentration 50) 5 to 10 times lower than that of the synthetic pesticides Chlopyrifos, Carbosulfan and Deltamethrin. In addition, [24] working on the doseresponse of clove oil in greenhouses showed that it reduced the population of *Meloidogyne incognita* to concentrations ranging from 0.1% to 0.3%. With respect to citrus extracts, [25] have reported the efficacy of extracts of Citrus sinensis on the root-knot nematodes *Meloidogyne* spp. in addition essential oils have been identified in citrus peels have shown bionematicide activity [26]. As for the FERTISAIN bionematicide (based on *Trichoderma harzianum* (3% Gamma lactone from *T. harzianum* extracts containing in addition 1% citrus terpene oil, 0.5% clove oil, 2% Magnesium Oxide, 0.1% manganese and 0.1% Zinc), it also contains components of the NEMATISAIN bionematicide. These include citrus and clove-based extracts in addition to trace elements. The fungus *Trichoderma harzianum* allowed a good control of the main parasitic nematodes of tomatoes, mainly species belonging to the species of *Meloidogyne* spp. and our results corroborate with those of several authors who have shown the efficacy of *Trichoderma harzianum* on *Meloidogyne javanica* ([27; [28]; [29]). VELUM Prime 400 nematicide (Fluopyram 400g/L) has been shown to be effective in reducing the populations of the endoparasite nematodes *Meloidogyne* and Pratylenchus during the sugarcane development cycle. Our results are consistent with those of several authors who noted the efficacy of Fluopyram 400g/L on nematodes in this group ([30]; [31]). Indeed, Fluopyram is known to be very active at all stages of the nematode development cycle by acting on the respiratory chain complex to block the production of energy by the nematodes. Fluopyram has been shown to be effective in suppressing plant root infestation by *M. incognita* because it disrupts chemoreception at the time of nematode penetration into host plant roots [32].

Effect of Bionematicides and Chemical Nematicide on Tomato Yield Components and Yield

In general, the bionematicides and the chemical nematicide improved tomato yields by 35% compared to the untreated control, which demonstrates the effectiveness of these products against tomato parasitic nematodes and in tomato productivity. In addition to the trace elements present in bionematicides, the importance of which in plant growth is known from an agronomic point of view, several authors have shown the effectiveness of synthetic bionematicides and chemical nematicides on tomato yield. Indeed, [33] have been shown that the triterpene compounds in neem extracts inhibit the nitrification process and increase the amount of nitrogen available, promoting plant development and increasing fruit yield and size. [17] also observed an increase in tomato fruit yield in plots treated with neem seed derivatives. As for the efficacy of *T. harzianum*, [34] noted improved plant development and an increase in fruit yield and fruit size as observed in the present study. This could be attributed to the release of growth-promoting substances or the production of toxic metabolites that inhibit nematodes and exclude other harmful microorganisms [35]. In addition, *Trichoderma* has been proven to contribute to tolerance to stress conditions by improving root development. It participates in the solubilization of inorganic nutrients. Thus, roots colonized by *Trichoderma* require less man-made nitrogen fertilizers [36]. Our results are also similar to those of [37] who observed increased growth and yield of tomato, soybean, tobacco, and pepper in potted and field experiments treated with *Trichoderma*. For VELUM, yield increases have been associated with the application of Fluopyram in the control of nematodes [38]. Our various pre-extension tests of the VELUM nematicide have indeed shown high yields related to the use of this nematicide in the control of tomato parasitic nematodes ([39]; [40]).

CONCLUSION

Bionematicides (NEMATISAIN and FERTISAIN) and chemical nematicide (VELUM) were effective in the control of tomato parasitic nematodes. The efficacy of the main components of the bionematicides including neem extracts, citrus extracts and clove extracts was observed in the two (2) bionematicides that contributed to the increase in tomato yields. As for *Trichoderma harzianum*, it has allowed a better development of the plant's root system and an improvement in tomato productivity. The VELUM chemical nematicide has improved tomato yields to levels comparable to bionematicides, which are proving to be an alternative in the fight against tomato-parasitic nematodes.

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