



## Assessment of the Effect of Different Varieties of Tomato and Hot Pepper on Nematodes Attack in Senegal

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### Abstract

Tomato and hot pepper are very susceptible to nematodes. The Parasitic Plant Nematodes (PPN) are controlled using chemicals or resistant varieties. This study focuses on appraising the resistance levels of local and American varieties through the plant characteristics (growth, biomass) and the nematodes metrics (gall index, population). For each crop, three American varieties were tested to ascertain their resistance to the nematodes in Senegal using the following plant materials and protocol. American tomatoe varieties are as follows: Small Fry, Jet Setter, and Celebrity. The hot pepper American varieties include Charleston Bell, Carolina Cayenne, and Carolina Wonder. All of these were provided by the Entomology and Nematology Department and Cooperative Extension Service at the University of Florida. They were compared with highly susceptible Senegalese local varieties which were Roma

and Orbit for tomato and Safi for hot pepper. The test was conducted in pots containing sterilized sand of dune and placed in a shelter in a completely randomized design. There were 200 second stage juveniles (J2) of *Meloidogyne* inoculated in each pot thirteen days after transplantation. After fifty days, plants were uprooted to collect agronomic and nematological parameters. Results for tomato showed, in general, better growth from Roma than the other American tomato varieties. Celebrity offered possibilities of reducing *Meloidogyne* population, while Small Fry and Jet Setter's growth were not affected by *Meloidogyne* populations. Safi, which is the local hot pepper variety, displayed the poorest growth. Carolina Cayenne variety also performed better than Safi in heavy infestations. Analysis of *Meloidogyne* J2 final population data (in the soil and root) showed a significant difference between Small Fry and Celebrity for tomato. As for hot pepper, Carolina Cayenne performed significantly better than Carolina wonder and Safi. This opens up further research opportunities on the impact of *Meloidogyne* nematode genus and the agronomic parameters.

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**Keywords:** *Meloidogyne*, Variety, Tomato, Hot Pepper

## I. Introduction

Agriculture occupies an important place in Senegal where 70% of the population resides in rural areas and grows cereals, legumes, and vegetables. Vegetable crops are especially important given their profitability, nutritional enhancement of traditional dishes, and the exports leading to more currency availability (Autissier, 1988). Senegalese vegetable production, estimated at 1,083,399 tons in 2017, is dominated by onion (400,000 tons). This is followed by potato (118,783 tons), cabbage (76,116 tons), sweet potato (72,000 tons), industrial tomato (70,000 tons), and cherry tomato (68,000 tons) (Direction de l'Horticulture, 2017). Originating from South America, tomato (*Solanum lycopersicum*) and hot pepper (*Capsicum frutescens*) belong to the Solanaceae family and Scrophulariales order. Tomato cultivation can be impeded by parasitism in equatorial regions or by the heat in Sahelian areas. On the other hand, hot pepper is cultivated the whole year in tropical areas. Tomato is the second most important vegetable in Senegal (Diallo et al., 2017; Sene et al., 2020). The importance of tomato and hot pepper is due to their content of minerals, antioxidants, and vitamins that are indispensable for the growth and proper functioning of human organs (Glodjinon et al., 2020). Tomato and hot pepper are susceptible to Root-Knot Nematodes (RKN) that decrease the root system. Also, they yield or Moreover, RNK annihilate the beneficial interactions with soil microorganisms (Diaw et al., 2019). Nematodes are the most abundant

multicellular organisms on earth and are responsible for important yield losses due to buildup of inoculum of the nematode and repeated cultivation of same cultivars in the same land every year (Hussain et al., 2016). Even if they form a homogenous group with regard to their characteristics, nematode species vary in function of the species and the environments they colonize (Morand, 2002). Parasitic plant nematodes are cylindrical worms invisible to the naked eye. These worms are generally 300 to 1,000  $\mu\text{m}$  in length and 15 to 35  $\mu\text{m}$  in width, but occasionally measure up to 3 mm. Nematodes possess an external cuticle containing the digestive system, the protractile stylet, and the reproductive system (Esser, 1971). Plant nematodes account for over 4,100 species—Thus, they falling into three groups. which These groups are subdivided in two sub-groups each (Poveda et al., 2020), i.e., sedentary nematodes that remain in the host plant and migratory nematodes that leave the host plant at a given time in their life (Ritter, 1971). Based on the feeding habits, the three groups are:

- Endoparasites which lay eggs inside the plant tissues are mostly found in the roots or in the leaves (for certain species) and the stems or other underground organs with a stem-like botanical structure.
- Semi-endoparasites which deeply fix themselves to the roots and lay eggs outside the host plant.
- Ectoparasites which remain in the soil outside the root they feed on using their stylet.

About thirty species of nematodes mainly belong to the genera *Meloidogyne*, *Helicotylenchus*, and *Scutellonema* parasitize crops and are from twenty different genera in Senegal (Netsher, 1970). *Meloidogyne*, which is known as the root-knot nematodes, are sedentary endoparasitic nematodes belonging to the Nematoda class, Secernentea subclass, Tylenchida order, Tylenchoidea Super family, and Meloidogynidae family. They are considered as one of the most damaging nematodes to crops worldwide and are ranked in the top five plant pathogens (Koenning et al., 1999; Mukhtar, 2018).

Due to the importance of plant nematode damages, which accounts for \$157 billion in global agricultural losses per year (Abad et al., 2008), highly effective but hazardous nematicides such as methyl bromide and 1,3 dichloropropene have been used as soil fumigants in the past century (Laquale et al., 2015). However, they are mostly crop specific and can be phytotoxic to certain crops (Morris et al., 2016; Oka et al., 2012). Moreover, environmental and human health concerns spur the withdrawal of most of these nematicides from the market. This has led to the search for alternative strategies (Ghorbani et al., 2008; D'Addabbo et al., 2010) such as soil solarization (Basallote-Ureba et al., 2010), phytochemical compound nematicides, semisynthetic phytochemical derivatives (Chitwood, 2002), fertilizer application in split doses (Benjamin et al., 2020); composts based on local vegetation such as

*Calotropis procera*, *Crotalaria juncea* (Sall et al., 2020); biological control agents such as *Trichoderma citrinoviride* (Fan et al., 2020); and resistant plant varieties. However, the tomato and pepper breeding programs offer few cultivars with root-knot nematode resistance (Maquilan et al., 2020). The Nematology laboratory of The Senegalese Agricultural University (ENSA) has carried out research on the resistance of American tomato and hot pepper varieties to the nematodes of *Meloidogyne* genus in local conditions. The main objective of this research is to review the susceptibility of these American varieties with regard to the local varieties that are susceptible to the *Meloidogyne* genus which affects more than 2,000 species belonging to 100 families (Yue et al., 2021). Specifically, it intends to assess the following: (1) plant growth during the vegetative cycle, (2) aboveground and root biomass, (3) the root-knot index, and (4) the final population of *Meloidogyne* nematodes (in the soil and root).

## II. Materials and Methods

To assess the effect of local and American varieties of tomato and hot pepper, the following materials and methods were used:

### 2.1. Plant Materials

The American varieties are supposed to be resistant, while the local varieties used as control are supposed to be sensitive to the nematodes.

#### 2.1.1. Tomato Varieties

The tomato varieties used in this study were Small Fry, Jet Setter, Celebrity (USA), and two local varieties (Roma and Orbit). Roma is a precocious and productive variety that resists *Fusarium* and *Verticillium*. The fruits, which are medium-sized and spherical, contain few seeds. Jet Setter is a heat-tolerating variety that produces medium-sized and round fruits. The fruit peel is thicker than the other varieties, which makes the Jet Setter variety less prone to the cracks. Celebrity is characterized as a semi-bush that produces two vegetative branches before any raceme. Small Fry is an upright small plant and thick peel fruits. This precocious variety bears high temperature. Orbit is a local variety that is deemed sensitive to *Meloidogyne* nematodes.

#### 2.1.2. Hot Pepper Varieties

The Charleston Bell, Carolina Wonder, Carolina Cayenne (USA), and Safi (local variety) pepper varieties were used.

Safi is deemed to be a susceptible variety. Wonder and Charleston Bell are two new cultivars sharing many similarities. The plants usually grow in a compact way and the cycle lasts 63 to 70 days. The results of two sets of

experiments conducted in the laboratory of vegetables in USA in 1996 showed that the plant and fruit characteristics of Carolina Cayenne and Carolina Wonder are practically identical. Local varieties of tomato (Roma and Orbit) and hot pepper (Safi) are deemed to be sensitive to the Meloidogyne genus.

**2.2. The Experimental Design**

The experiment was conducted using a completely randomized design with one treatment (variety) and five replications. In total, 45 pots were used of which 25 were for tomato and 20 were for hot pepper. The pot weight after filling was 2 kg and 2.5 kg for tomato and hot pepper respectively. Each pot was labelled and kept according to the following designs.

Crop	VARIETIES	CODE
Tomato	Small fry	V1
	Jet Setter	V2
	Celebrity	V3
	Roma (local, sensitive)	V4
	Orbit (local, sensitive)	V5
Hot pepper	Charleston bell	V1
	Carolina wonder	V2
	Carolina cayenne	V3
	Safi (local, sensitive)	V4

V4	V4	V5	V1	V1
V1	V3	V4	V1	V3
V4	V5	V4	V5	V2
V2	V2	V3	V2	V3
V1	V5	V5	V3	V2

V4	V1	V3	V4	V1
V2	V2	V2	V3	V2
V4	V1	V3	V2	V4
V3	V4	V1	V4	V1

\* V: variety

**Figure 1.** Experimental Design for Tomato (Left) and Hot Pepper (Right)

**2.3. Data Collection**

**2.3.1. Nursery, Sowing, and Management**

The seeds were sown in seedling trays and placed in a secured location. Thereafter, daily watering was conducted. After 24 days in the nursery, the seedlings were transplanted into pots filled with sand dune sterilized in a heat chamber at 160° for 24 hours to obtain sterile substrate. The watering was conducted twice a day at 8 am and 5 pm.

**2.3.2. Inoculum Preparation and Inoculation**

Guava roots infested with Meloidogyne nematodes were cut and put on 100µm-sieves, which was soaked for 7 days. The roots were afterwards removed and the solution was collected in a test tube for nematode counting. The plants were inoculated with 200 nematodes using a graduated pipette containing 5 cc of the solution.

### **2.3.3. Agro-Morphological Observations**

Observations on plant height from the collar to the last leaf's insertion were conducted up to 49 days after transplantation. Afterwards, the plants were harvested and the following parameters were also measured: aboveground biomass, root biomass, root-gall indices, and number of *Meloidogyne* individuals. After careful uprooting of plants, the aboveground and root parts were weighted and separated. Using the root-gall indices, the infestation severity degree was assessed on a scale of 0 to 10 per Zeck (1971).

### **2.4. Analysis of *Meloidogyne* Populations**

The nematodes were extracted according to the simplified Baerman funnel method (Hooper, 1990), which consist of adding water in 250 cc of soil in a plastic bucket. The soil solution was allowed to settle for 15 minutes. The supernatant was filtrated with a three-level sieve with decreasing meshes size of 2mm, 100µm, and 40µm.

### **2.5. Soil and Root Sampling**

For each pot, samples of 250g of soil and 2g of roots were collected and labelled. Therefore, 25 and 20 soil samples were collected for tomato and hot pepper respectively. The same number of root samples was also collected for tomato and hot pepper. Nematodes were extracted using the following methods:

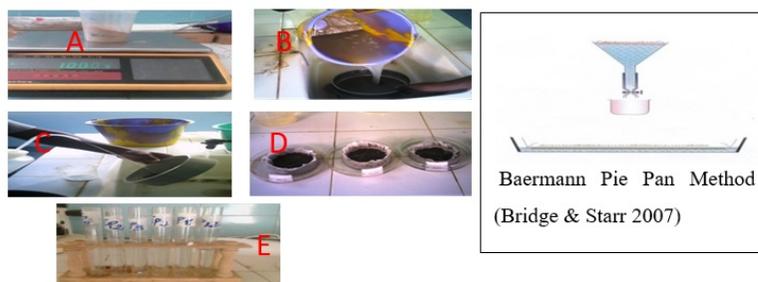
#### **2.5.1. Soil Nematodes' Extraction**

Soil nematodes were extracted using the elutriation method of Seinhorst (1962), which is based on the settling of particles in water. A 250 cm<sup>3</sup> volume of soil was suspended in a column of water. To separate the particles, ascending water current was distributed through the column for 30 minutes to raise and collect the nematodes in a bucket. The suspension (water, nematodes and debris) was filtrated using four sieves with a mesh size of 50 µm. The refusals from sieving were poured in a sieve that had a mesh size of 100 µm and was covered by two pieces of tissue. The filtrate was placed in a Petri dish filled with water. Nematodes present in the Petri dish were counted after 48 hours.

#### **2.5.2. Root Nematode Extraction**

Roots were washed under weak water current. A gall-index was assigned to each root system per Zeck (1971). Nematodes were extracted from the roots according to Seinhorst (1950). A certain mass of roots was placed on a coarse sieve immersed in an overfilled dish. The dish was exposed to a fog inside a chamber until the roots began to decay. Nematodes came out after that and were transported by the current. The suspension containing the nematodes

and vegetal debris was poured on a sieve covered with tissue after 7 days and 14 days. The suspension is purified the same way as for the soil elutriation.



**Figure 2.** Different Steps of the Nematode Extraction

The various steps of the nematode extraction are outlined as follows: A) soil sample weighting, B) sieving of the soil solution with the 40  $\mu\text{m}$  mesh, C) recovery of the sieve residues, D) setting the sieve residues down on 50  $\mu\text{m}$ -sieve for the active passage of nematodes, and E) recovery of the sieve residues and collection of the extracts in test tubes (Karmadini, 2007).

## 2.6. Identification and Counting

Nematode identification and counting were carried out on an aliquot of 5 cc of the extract (5/25). Sampling was done using a pipette and the number of individuals was divided by the pot weight. Binocular magnifiers and optical microscopes were used to evaluate morphological characters and identify different genera. Identification keys were used when needed. The number of nematodes was counted in each root sample of 2g. The number of nematodes corresponding to the root mass was deduced from the number of individuals per sample for each variety and for each replicate.

## 2.7. Data Analysis

The processing and the graphical representation of the raw data were conducted in Microsoft Excel. The analysis of variance and the Student-Newman-Keuls test (at 5% level) were performed using the STATVIEW program.

## III. Results

### 3.1. Tomato Plant Height

The tomato varieties such as Small Fry, Jet Setter, and Celebrity were taller at transplantation than the local varieties of Roma and Orbit. However, Roma prevailed over Small Fry, Jet Setter, Celebrity, and Orbit beyond the 7<sup>th</sup> day after transplantation. The growth of the tomato varieties, i.e., Small Fry, Jet Setter and Celebrity, slowed down 14 days after transplantation.

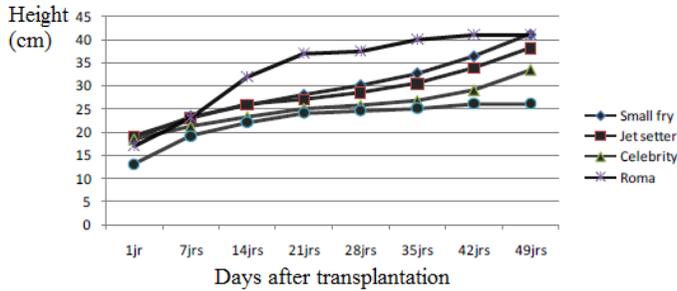


Figure 3. Tomato Plant Height after Transplantation

### 3.2. Hot Pepper Plant Height

The hot pepper variety, i.e., Carolina Cayenne, showed the best growth. This was followed by Carolina Wonder and Charleston Bell. Safi, which is the local variety, lagged behind from the transplantation up to the 49<sup>th</sup> day. Carolina Cayenne grew faster than the other varieties one week after transplantation. Interestingly, this trend was maintained up to the 14<sup>th</sup> day.

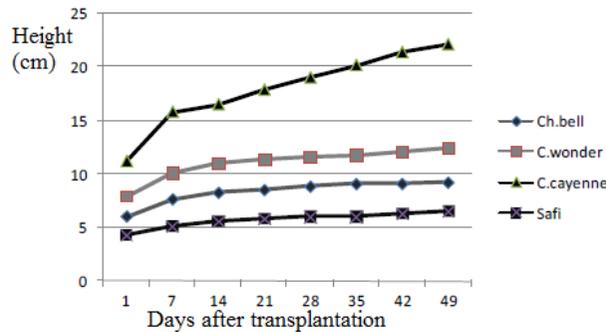


Figure 4. Hot Pepper Plant Height after Transplantation

### 3.3. Aboveground and Root Biomass of the Tomato Varieties

Roma variety had the most important aboveground biomass (18g), followed by Celebrity (13.2g), Jet Setter (12.8g), Small Fry (9.6g), and Orbit (7.6g). Nonetheless, Roma and Celebrity had the greatest root biomass at 6.4g each. Jet Setter had slightly less root biomass with 6g, while Small Fry and Orbit lagged behind at 4.8g and 4.6g respectively.

Table 1. Tomato Aboveground and Root biomass

Variety	Aboveground biomass	Root biomass (g)
Small Fry	9.6±03	4.8±09
Jet Setter	12.8±01	6±04
Celebrity	13.2±07	6.4±05
Roma	18±03	6.4±01
Orbit	7.6±01	4.6±03

### 3.4. Aboveground and Root biomass of the Hot Pepper varieties

In terms of plant height, Carolina Cayenne variety showed the greatest aboveground biomass at 5.3g, followed by Charleston bell (2.9g), and Carolina wonder (2.4g). Conversely, Safi variety had the lowest aboveground biomass at 0.72g and also had the lowest root biomass at 0.72g, which is behind the Carolina Wonder (2.4g) and Charleston bell (2.9g) varieties. The Carolina cayenne variety displayed the highest root biomass (3.9g).

**Table 2.** Hot Pepper Aboveground and Root biomass

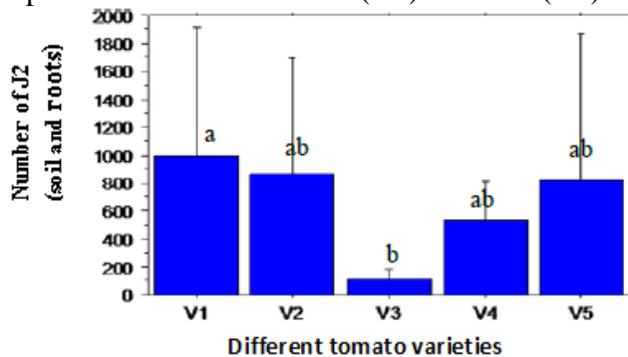
Variety	Aboveground	Root biomass
Charleston	2.9 ± 01	2.7±07
Carolina	2.4 ±05	2.8±01
Carolina	5.3±02	3.9±01
Safi	0.72±01	1.74±03

### 3.5. Final Population of Meloidogyne in the Tomato Soil and Roots

Among the tomato varieties, only Small Fry (V1) and Celebrity (V3) had significantly different levels of final population of J<sub>2</sub> Meloidogyne between the soil and the roots.

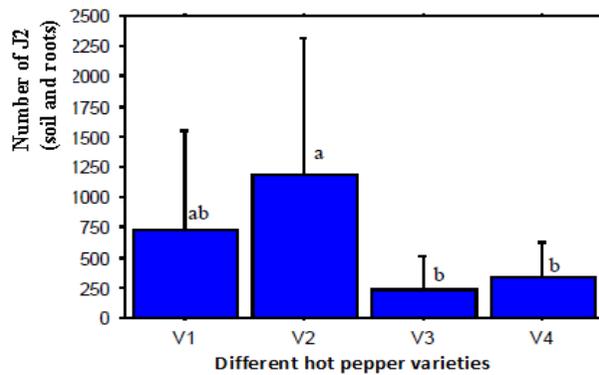
### 3.6. Final Population of Meloidogyne in the Soil and in the Hot Pepper Roots

The Carolina Cayenne (V2) variety showed a significantly larger population of final population of J<sub>2</sub> Meloidogyne between the soil and the roots when compared to Carolina wonder (V3) and Safi (V4) varieties.



*The Varieties with Same Letters are not Significantly Different*

**Figure 5.** Final Population of Meloidogyne in the Soil and the Tomato Roots



*The Varieties with Same Letters are not Significantly Different*

**Figure 6.** Final Population of Meloidogyne in the Hot Pepper Soil and Roots

#### IV. Discussion

It is well-known that the solanaceous crops are particularly susceptible to Meloidogyne nematodes (Abad et al., 2003). However, their reaction to the infection may vary greatly.

In this study, American tomato varieties such as Small Fry, Jet Setter, and Celebrity display good plant growth in the nursery compared to local varieties. However, the local variety, i.e., Roma, begins to perform better after the transplantation and even before the inoculation. Conversely, the other local variety, i.e., Orbit, displayed relatively modest growth compared to the American varieties.

Roma and Orbit had the lowest root-gall indices, while Small Fry and Jet Setter showed very high indices, indicating a greater susceptibility of these varieties to the Meloidogyne genus. These performances may also be due to the low inoculum rate (200) since previous studies have shown Roma susceptibility to nematodes despite an inoculum rate of 300 larvae per plant (Touré et al., 2019). The Meloidogyne nematodes appear to have an affinity with the Celebrity variety, which reveals that the root-gall indices are quite high and close to those of Small Fry and Jet Setter. However, the presence of the root gall did not impede the growth or result in greater aboveground biomass than root biomass. The root galling was shown to alter water and nutrient uptake by the root system, thereby causing a reduced height (Murad et al., 2020). The Celebrity variety may possess an adaptation mechanism which allows proper plant nutrition in spite of the nematode infection.

The final population of J<sub>2</sub> Meloidogyne is higher for Small Fry, Jet Setter, and Orbit. This shows that these varieties fostered the multiplication of the Meloidogyne genus, while Celebrity and Roma restricted their development. Previous studies showed a reduced final nematode population six weeks after inoculation of the *Solanum* spp with root knot nematodes

(Agyeman, 2016). This trend was explained by the interaction between the rootstock and the initial nematode population. The increased *Meloidogyne* population for Small Fry and Jet Setter did not hamper the development of the plants. Hence, their average height was greater than the height of Orbit which showed smaller *Meloidogyne* population. These findings are in line with those of Duponnois et al. (1997) who highlighted positive correlation between plant height, aboveground biomass, and nematode index in *Acacia* species. In contrast, low gall index/nematode population and important height have been reported in okra (Sani et al., 2021). The Orbit variety was more susceptible to the *Meloidogyne* genus than Small Fry and Jet Setter, while Roma appeared to show a certain level of tolerance. However, the Celebrity variety had a lower average height than Orbit in spite of the lower *Meloidogyne* population. This indicates that the strong presence of *Meloidogyne* in the Orbit plant roots had no negative effect on its growth. In other words, Celebrity is more susceptible to the presence of *Meloidogyne*. The Roma variety began to blossom 21 days earlier than the other varieties after transplantation. More so, yellowing, wilting, and leaf fall were observed on all the varieties.

American hot pepper varieties grew better than the local variety, Safi. In addition, Carolina Cayenne performed best, followed by Carolina Wonder, and Charleston Bell. This could stem from the presence of the “N” gene conferring nematode resistance to the Charleston Bell and Carolina Wonder varieties (Hu et al., 2020). The growth was rapid immediately after transplantation but slowed down after inoculation was conducted on the 14<sup>th</sup> day. Safi proved to be less susceptible to the *Meloidogyne* than the American varieties (Carolina Cayenne, Carolina Wonder and Charleston Bell) with a lower root-gall index. The Charleston Bell and Carolina Cayenne varieties had similar root-gall indices with a predominance of aboveground biomass over the root. The converse pattern (more root biomass than aboveground biomass) was observed with Carolina Wonder and Safi whose root-gall indices were different.

The highest final populations of *Meloidogyne* were observed on Carolina Wonder and Charleston Bell with a strong presence in the roots. Previous studies have reported higher infestation levels of *M. incognita* J<sub>2</sub> in ‘Charleston Belle’ than in ‘Carolina Wonder’ under greenhouse conditions (Aguilar et al., 2014).

Furthermore, revealing the smallest final populations of *Meloidogyne*, Carolina Cayenne and Safi varieties had the highest soil nematode populations. Carolina Cayenne and Carolina Wonder had the best growth, which indicates that the strong presence of *Meloidogyne* soil nematodes did not affect the growth. Safi hosted the lowest *Meloidogyne* population and experienced a considerably delayed growth. Considering the slow growth of Safi, it would be problematic to interpret its susceptibility to nematodes only

in reference to height measurements. This is because it was less infested with the *Meloidogyne* nematodes (Zeck, 1971). Yellowing, leaf fall, and wilt were observed during the growth process as in the case of the tomatoes. The Carolina Cayenne variety began to bloom the fourth week after transplantation while other varieties did not blossom yet. Although it was deemed susceptible to *Meloidogyne*, the two local tomato varieties (Roma and Orbit) and one local hot pepper variety (Safi) were relatively less susceptible than the American varieties under study. Roma performed better than Small Fry, Celebrity, and Jet Setter even though Celebrity variety offered more possibilities to reduce *Meloidogyne* populations. However, Small Fry and Jet Setter withstood the *Meloidogyne* attacks better than the local variety Orbit. Furthermore, Celebrity variety demonstrated unusual behavior that necessitates investigation of the relationship between *Meloidogyne* population and plant height. The high densities of *Meloidogyne* populations observed in most of the root samples indicate that these nematodes are endoparasitic (Ritter, 1971). At inoculation, nematodes entered the roots where they disturbed the plant's hydro-mineral nutrition (Sasser, 1979). The damage causes plant stunting which translates into qualitative and quantitative depreciation of the production as reported by Sardanelli and Ellison (2005).

## **V. Conclusion and Prospects**

This research evaluated American tomato and hot pepper varieties which is supposed to be resistant to the *Meloidogyne* in comparison with local susceptible varieties. Plants were grown in pots and inoculated the 13<sup>th</sup> day after transplantation. Weekly agro-morphological data were collected during the plant growth. Analysis of root gall and *Meloidogyne* final populations was performed in the lab on the 49<sup>th</sup> day after transplantation. Although regarded as a susceptible tomato variety, Roma appeared to show a better performance with an important aboveground and root biomass. Therefore, this shows a low root-gall index and a population of root nematodes higher than the soil population. The American variety celebrity recorded both the lowest root and soil nematode populations and was second to Roma in terms of biomass. With regard to plant height, Roma was followed by Small Fry. However, the latter variety was highly infested by *Meloidogyne* and had the highest root-gall index. Conversely, Safi performed poorly compared to American varieties. More so, its root-gall index and infestation level were the lowest. In order to better appraise the plant growth in function of *Meloidogyne* nematode infestation, future studies should consider non inoculated control varieties in addition to the American resistant varieties. In the same vein, field trials would enable the assessment of the different varieties in relation to nematode settling by staggered sampling on the 10<sup>th</sup>, 20<sup>th</sup>, 30<sup>th</sup>, and 40<sup>th</sup> day. Genetic analysis could shed more light on the gene characterizing these different varieties.

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