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Diagnostic of tomato Nematode in Daloa (Côte d'Ivoire)

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Abstract

Symptomatological studies were carried out in two tomato growing areas in Daloa to estimate and identify the associated nematode populations. Symptoms were assessed by visual observation. The soil and root nematodes were extracted by Bermann's method and identified by observing morphological characteristics. The symptomatological study showed the presence of symptoms of plants wilting, yellowing of the leaves as well as galls on the nematodes characteristic roots. The results also highlighted diversity within the nematode population that colonizes tomato in Daloa with four genera of nematodes. The genera *Tylenchus*, *Helicotylenchus, Partylenchus* and *Meloidogyne* were identified. The presence of the genus *Meloidogyne* in all plots shows that it is responsible for the yellowing symptoms associated with root galls. These nematodes are known for their action on the formation of galls on the roots of the tomato.

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Introduction

The tomato (*Lycopersicon esculentum* Mill), Solanaceae is considered as the first vegetable after potato and the second world food resource after cereals. Its culture is adapted to very varied culture conditions. Tomato production has continued to grow in recent decades around the world; from 48 million tonnes in 1978, it rose to 124 million in 2006 (Blancard *et al*., 2009). In Côte d'Ivoire, the tomato sector is booming with production increasing over time. For Faostat, production was estimated at 26,235 t in 2005 and rose to 40,306 t in 2018. This crop generates income and employment for vulnerable groups and for youth (Tujague-Gibourg and Moustier, 2009).

However, tomato cultivation is faced with many parasitic constraints. Root-knot nematodes of the genus *Meloidogyne* are among the most important pests of tomatoes (Bissadou, 2012). Symptoms from nematode attack are characterized by the appearance of galls on the roots and reduced growth of infected plants (Cunha *et al*., 2018). These pests, although less integrated in research programs, are among the most important causes of crop yield reduction. It is estimated that approximately 11% of global crop losses are caused by nematodes, resulting in a crop loss of several million tonnes each year (Gregory *et al*., 2017).

The damage caused by *Meloidogyne* spp. lead to significant production losses in areas with favorable conditions (Castagnone-Sereno and Djian-Caporalino, 2011). In Côte d'Ivoire studies have been conducted on these worms which reduce tomato production. Nematode damage is difficult to quantify because it affects production by direct and indirect action.

The fight against these terrestrial parasites should begin with the information about the diversity of the tomato parasitic nematodes. The present study aims to contribute to tomato production in Daloa region through the understanding of nematode species infested with tomatoes in the area under study. This will specifically involve the causes of the damage by these nematodes and the diversity of species on the tomato crop.

Material and methods

Study area

This experiment was carried out in Daloa, region of Haut Sassandra, located at 6°53 north latitude and 6°27 west longitude. Daloa is the capital city of the Haut-Sassandra region and is located about 141 km from Yamoussoukro, the political capital city and about 400 km from Abidjan, the economic capital. Sampling was made from October 2019 to January 2020 in two areas of Daloa: Djekro which is located 12 km from Daloa and the "archive" area 2 km from Jean Lorougnon Guédé University. In the Djekro area with high tomato production, eight (8) tomato plots were sampled against two (2) in the "archive" zone.

Biological material

The biological material consists of infected tomato plant and tomato growing soil collected from the Djekro plots and the "archive" area from November 2020 to January 2020.

Method

Sampling

Symptoms were assessed by visual observation of the tomato plants. Plants showing symptoms were counted by symptom type and by ridge. The assessment was carried out throughout the plot.

Then, the plants showing the symptoms were thoroughly dug up with a knife. The growing soil around the unearthed plants was collected and put in sachets and taken to the laboratory for various tests. The incidence was assessed by plot and by type of symptom. Symptom evaluation was carried out during the vegetative phase (one to two weeks after transplanting the tomato plants) and during the fruiting phase.

Extraction of nematode

The modified Baermann method or the plate method, or the Whitehead plate method was used for the extraction of nematodes (Coyne *et al.*, 2010). This method provides a reliable estimate of nematodes in soil, roots, seeds and plant tissues while being easily repeatable.

The device consisted of a saucer in which a large mesh sieve was placed. Then a filter paper was placed in the bottom of the sieve. For nematode extraction, soil and root samples were treated differently. The samples collected in each plot were mixed to obtain a composite sample and the resulting mixture was sieved to remove roots, stones and various debris. A 100mL soil sample was taken using a beaker for extraction. The root samples were rinsed with tap water and cut into small pieces with a knife, weighed and crushed in a blender. The collected soil and root samples were placed in the sieve covered with filter paper. Using a squeeze bottle, the water volume needed to moisten the sample was gently poured between the rim of the saucer and the sieve.

The preparation was stored in the dark under laboratory conditions for 48 hours. After the 48 hours, the water suspension contained in the saucer was collected in a beaker. The solution was left for 24 hours and was homogenized with a pipette and then mounted between slide and cover slip for identification and counting of nematodes by microscopic observations.

Identification of nematodes

After extracting the samples, the solutions obtained were mounted between slide and coverslip for the various observations. The density of nematodes was assessed by counting individuals observed in root and soil solutions. For the identification of nematodes, the parameters observed are: length, width of the body and length of the tail. Identification was performed using identification keys from Mekete *et al.* (2012).

Data analysis.

The incidence of symptoms was assessed using Excel software and statistical version 7.1.

Results and discussion

Results

Symptomatology

The surveys carried out on the plots made it possible to detect two types of leaf symptoms: wilting of the entire plant which sometimes ends up dying and yellowing of the leaves. Both types of symptoms were found in the different study plots (Fig. 1A and B). Symptoms of galls have also been observed on the roots of some plants. In some cases, the entire root system is covered with galls. These root galls have been observed in most plants showing symptoms of yellowing. However, in terms of wilt symptoms, few plants showed galls on their roots (Fig.1C).

Fig. 1. Symptoms on tomato plants (A) wilting; (B) yellowing (C) root galls.

Assessment of the incidence of leaf symptoms in tomatoes

The incidence varied depending on the type of symptoms and the plots collected (Table 1). Overall, the incidence of yellowing was significantly higher (P = 0.00 <0.05) than that of wilt. The mean incidence ranged from 14.47 to 4.02% for yellowing and from 3.59 to 1.49%. At the plot level, the incidence of symptoms also varied from plot to plot (P = 0.00 <0.05). Thus, the incidence of yellowing made it possible to classify the plots into four groups with plots 7 and 8 having the highest average incidences.

Nematode density

The density of nematodes varied depending on the type of sample and the plots visited (Table 2). Thus, no nematode was recorded in plots 9 and 10 at both soil and root level. No nematode was observed in the solutions of plots 3 and 4.

Table 1. Incidence of wilting and yellowing symptoms according to plots.

Plots	Incidence of yellowing (%)	Incidence of wilting (%)
1	8.86 ab	2.47 EF
2	7.08 bc	1.49 F
3	10.18 ab	1.63 E
4	6.06 bc	3.59 CD
5	5.12 cd	3.22 CDE
6	9.00 ab	3.18 CDE
7	14.47 a	1.96 DEF
8	13.24 a	2.64 EF
9	4.02 cd	2.39 EF
10	5.72 cd	2.60 EF

Means followed by the same letter are not significantly different from one another at LSD test.

Table 2. Density of nematodes in soil and root samples from plots.

Plots	Soil (50 g)	Root (50 g)
1	345	337
2	191	306
3	0	245
4	0	135
5	209	760
6	120	532
7	150	131
8	524	170
9	0	0
10	0	0

Identification of nematodes

Four genera of nematodes were identified from soil and root samples: *Meloidogyne* sp., *Pratylenchus* sp., *Helycotylynchus* sp. and *Tylenchus* sp. *Meloidogyne* sp. is characterized by a slender tail and a flattened head (Fig. 2).

This species was mainly noticed in all the plots. *Helycotylynchus* sp. with body spiral habitus and rounded tail (Fig. 3). *Tylenchus* sp. is characterized by a flattened head and a tapering tail and *Pratylenchus* sp. with rounded tail (Fig. 4).

Fig. 2. Morphological characteristics *Meloidogyne* with (A) entire body, (b) Tail and (c) Head with stylet.

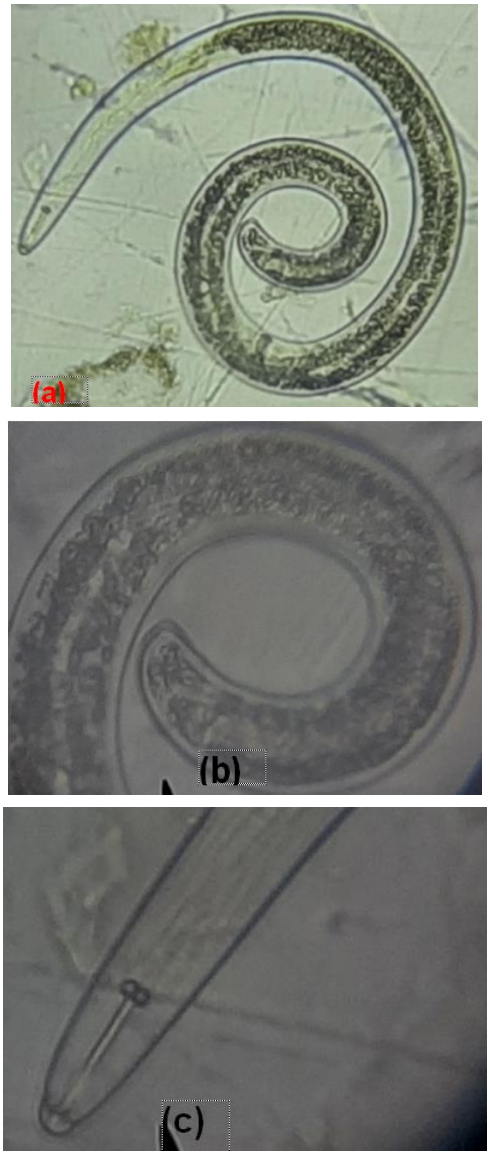

Fig. 3. Morphological characteristics *Helicotylenchus* sp. (a) body with (b) round Tail and (c) Head

Fig. 4. Morphological characteristics of (A) *Pratylenchus* sp. and (B) *Tylenchus* sp.

Discussion

Surveys carried out in different tomato plots (*Lycopersicon esculentum* Mill) showed different types of symptoms: wilting of the plant, yellowing of the leaves as well as galls on the roots of tomatoes. These symptoms were observed in all the tomato plots. The results on the incidence of symptoms highlight that at the level of yellowing the incidence during the vegetative phase is high compared to the fruiting phase. This reveals that the vegetative phase is sensitive. The wilting gives a low incidence at the level of the vegetative phase and the fruiting phase. The low incidence of wilting in the two phases shows that the tomato crop is not very sensitive to this disease, or that the prospected areas are not

favorable for the development of the disease. These wilting symptoms can be due to bacteria. Indeed, *Ralstonia. solanaceaum* is a soil-born bacterium responsible for the wilting of nightshades (Guinard, 2012).

The bacteria cause damage to many crops, including apples and tomatoes. The results also indicated that the incidence of yellowing in both phases is higher compared to that of wilting. The high incidence of yellowing shows that the tomato crop in this area is more susceptible to the disease. The results also revealed the presence of galls on the roots, characteristic of nematode attack. These root galls are associated with plants with yellowing leaves. The yellowing observed on tomato leaves is due to nematodes' attack. These results are in line with those of Mokrini (2016) who posited that in the region of Souss-Massa in Morocco, the attack of *Meloidogyne* manifests itself on the aerial parts by the yellowing of the leaves and the wilting of the plant. For authors like Coyne *et al*. (2010) the parasitism of *Meloidogyne* does not cause the appearance of specific symptoms on the upper part of the host plant, which makes the task of detecting them very challenging. The yellowing of the leaves observed in our work would therefore be a secondary impact of the attack of the roots by nematodes. These phytophagous nematodes cause the formation of galls on the roots modifying the absorption of water and nutrients by the plant and thus create water and mineral stress (Cunha *et al*., 2018). A high incidence of yellowing was observed in plots 7 and 8 located in the Djekro area is an intensive tomato production area. This pathology could negatively impact tomato production in Daloa.

The morphological parameters of the nematodes observed are as follows: body and tail length showed a variety of tomatoes nematodes attack. The results identified four genera of nematodes, namely, the genus *Meloidogyne* sp., *Helicotylenchus* sp., *Pratylenchus* sp. and *Tylenchus* sp. of these genera of nematodes identified, *Meloidogyne* sp. and *Pratylenchus* are endoparasite while *Tylenchus* sp. and *Helicotylenchus* sp. ectoparasites (Kouamé *et al*., 2018). The presence of *Meloidogyne* indicates that the galls observed are due to the infestation of this nematode. The nematodes of the *Meloidogyne* genus are known for their action on the formation of root galls by the secretion of substances which cause the production of giant cells. Also *Pratylenchus* genera are called root lesion nematodes. They cause necrosis of the root cortex of plants thus causing lesions or cavities in this tissue that will subsequently be colonized by secondary pathogens such as fungi and bacteria (Villain, 2000). Root galls are therefore caused by attack by nematodes of the genus *Meloidogyne*. The genus *Meloidogyne* or nematodes with knots or nematodes of the knotty roots (Root-knot nematodes) are roundworms elongated in spindle, not segmented with bilateral symmetry. This genus includes more than 90 species which are responsible for about 5% of the overall yield losses of food production (Djian-Caporalino *et al*., (2018).

In our study, *Meloidogyne* sp. has been identified, unlike the work of Berrabah *et al*. (2014), who identified in addition to *Meloidogyne* other nematode species on tomatoes. They showed that the populations of *Meloidogyne*, *Helicotylenchus*, *Tylenchorhynchus* and *Xiphinema* increased the practice of growing tomato which is their main host. The presence of *Tylenchus, Pratylenchus* and *Helicotylenchus* in tomato growing areas shows that they are secondary parasites or are also responsible for the symptoms observed in tomato leaves. Indeed, faunistic studies carried out in many African countries including the Ivory Coast, showed that *Meloidogyne*, *Pratylenchus* and *Helicotylenchus* are very polyphagous genera of phytoparasitic nematode observed on all cultivated plants (Fortuner 1987; Gnonhouri and Adiko, 2005). Also, Kouamé *et al*. (2018) also identified a diversity of nematodes including the genera *Meloidogyne*, and *Helicotylenchus* and *Tylenchus*

in Ivorian sugar complexes. The quantity of nematodes in the soil is less than that of the root. The genus *Meloidogyne* is present in soil and in roots while *Helicotylenchus* and *Tylenchus* have been found in soil. They are ectoparasitic nematodes that are found mainly in soils and feed on plant roots. This shows their high density in the soil. Nematodes were not in all soil samples. This could be explained by the fact that the proliferation of certain genera of nematodes is influenced by parameters such as the rainy season and the type of soil (Cadet and Debouzié, 1990). Samples were collected at the beginning of the dry season, with some plots located on the edge of the lowland. This could influence the abundance of some genus and the absence of *Tylenchorhynchus* and *Xiphinema* which are hosts of tomato.

Conclusion

The tomato (*Lycopersicon esculentum* Mill) from the Solanaceae family is one of the most popular vegetables around the world. On the other hand, its culture has been limited and weakened by diverse of microbial diseases from different origins. Nematodes are among the parasites that most threaten tomato growing. The results of our work showed the presence of symptoms characteristic of nematodes in the plots visited. These symptoms are characterized by wilting of plants, yellowing of leaves, and galls on the roots. Morphological characters have shown that there is diversity within the populations of nematodes that infect tomatoes. Thus, four genera of nematodes have been identified, namely the endoparasites *Meloidogyne* and *Pratylenchus* and ectoparasite *Tylenchus,* and *Helicotylenchus*. In all plots collected, the nematodes were dominated by the genus *Meloidogyne* sp.

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