



## Dynamics of phytonematodes according to treatments aqueous extracts- based (*Allium sativum* and/or *Azadirachta indica*) and the host plant (*Abelmoschus esculentus*)

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### Abstract

A study on the dynamics of phytoparasitic nematodes as a function of treatments aqueous extracts- based of *Allium sativum* and/or *Azadirachta indica* but also of the host plant, okra (*Abelmoschus esculentus*), was carried out at the “Institut Supérieur d’Agriculture et d’Entrepreneuriat” (ISAE) test station, from June to September 2020. This study was undertaken as part of the sustainable management of bio-aggressors. We were able to develop two biocides, one based on *Allium sativum* and the other on *Azadirachta indica*. From 68 soil samples we extracted 1958 specimens among which 904 are identified as free nematodes and 1054 as parasitic plant nematodes; phytonematodes of the Order of Rhabditida are more numerous with a prevalence of 53.6%. Our study pointed out that the biocides used have no effect on the phytonematodes encountered or on gumbo, but also that gumbo has no effect on these phytonematodes. However, our results show that okra is resistant to rhabditic phytoparasites found in the plot. This type of study is being conducted for the first time on phytonematodes in Senegal.

**Keywords:** Dorylaimida, nematofauna, okra, rhabditida, treatments

### Introduction

In Africa, food security has long been seen in terms of the amount of food available to different segments of the population. The achievement of this objective is not only based on produced and marketed quantities, the capacity of markets to import and the constitution by States of safety stocks but also the accessibility of consumers to a healthy and diversified diet Dugué *et al.* (2017) [2]. Similarly, health and nutritional quality of food is increasingly seen as a condition for food security and more generally for achieving the objective of sustainable development (Dugué *et al.*, 2017) [2]. In the achievement of food security, market gardening products have an important place (Grubben *et al.*, 2014) [4]. Moreover, with the development of the use of pesticides in horticulture for three decades, informed consumers are expressing doubts about the sanitary quality of vegetables and fear for their health. In response to this, consumer associations, development organizations and researchers are advocating a change in the management of vegetable production systems based on the principles of agro-ecology, from which the agro-ecological transition (Dugué *et al.*, 2017) [2]. Currently, the shift from chemical nematicides to organic nematicides from plant-based substances is taking place worldwide (Sithole., 2021) [10]. According to the same authors, treatments with increasing concentrations of plant extracts based on *Vernonia colorata*, *Searsia lancea* and *Pelargonium sidoides* suppress egg mass hatching after 72 h. Spraying 15% water-soluble hydrogenated fish oil also reduces nematode infestation (Kumar and Yadav, 2019) [8]. According to these, corn oil, cottonseed oil, groundnut oil and soybean oil reduce the number of ectoparasitic nematodes. *Azadirachta indica* has been shown to have a direct immobilizing effect on root-knot nematodes (Gravanis *et al.*, 2011) [5]. Based on this observation, we hypothesized that the aqueous extracts of *Allium sativum* and *Azadirachta indica* are effective in

reducing nematodes. Moderate concentrations of *Azadirachta indica* provide significant results to suppress the effects of root-knot nematodes on cowpea yield and growth (Nwankwo *et al.*, 2016) [9]. It is in this perspective that this study was undertaken to contribute to the sustainable management of bio-aggressors including phytophages nematodes. It aims to characterize the population of nematodes infested with okra and to evaluate the effect of aqueous extracts of *Allium sativum* and *Azadirachta indica* on this nematode population.

### Materials et Methods

#### 1. Presentation of the study area

The study was carried out at the ISAE school field at the Cheikh Anta Diop University of DAKAR from June to September 2020.

#### 2. Materials

##### 2.1 Plant material

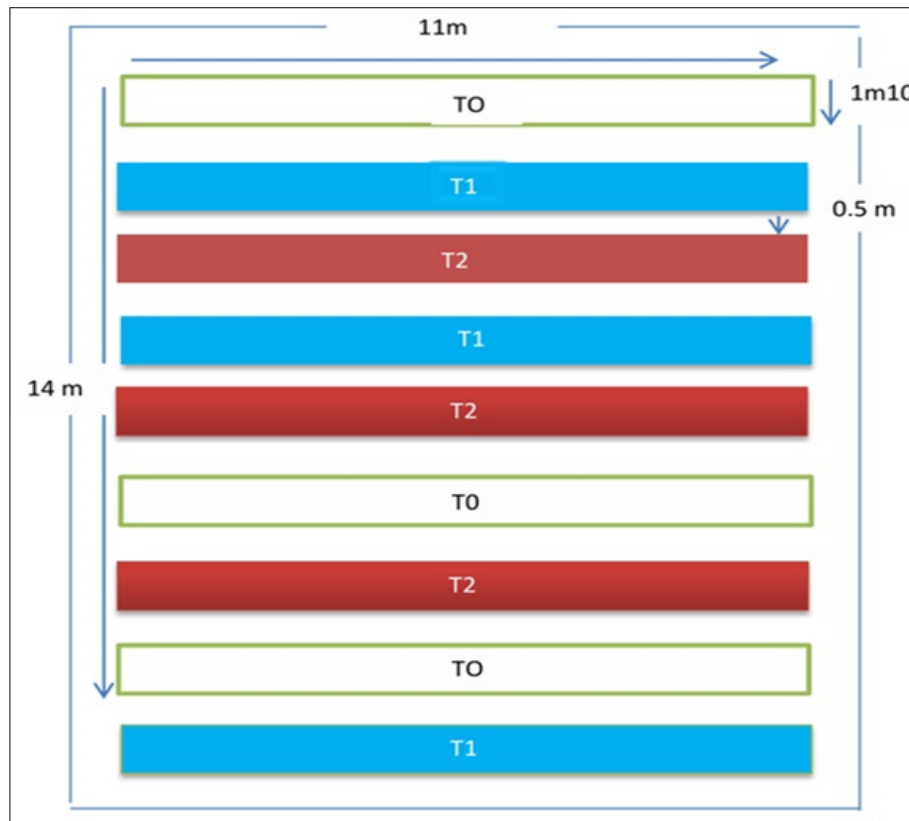
The plant material used was certified okra seed of the variety clemson spinless which is a variety imported from the United States. Seeds come from Niayes Sarraut, located in the city of Dakar, in front of Kermel market. There are also leaves of *Azadirachta indica* and pods of *Allium sativum* which were used for the preparation of phytosanitary treatments.

##### 2.2 Animal material

It concerns nematodes extracted from soil.

#### 3. Experimental setup

For our test, we used a model of completely randomized blocks with 2 types of treatments and 3 repetitions. Each plot had a dimension of 11m long and 1m10 wide, corresponding to an area of 12.1 m<sup>2</sup> per plot. There were 9 plots separated by 0.5 m aisles (Figure 1).



**Fig 1:** Mass plan of the experimental setup

**T0:** control plot.

**T1:** plot treated with maceration of *Azadirachta indica* leaves.

**T2:** plot treated with maceration of *Allium sativum* pods.

**4. Preparation of aqueous extracts**

Two kilograms of fresh leaves of *Azadirachta indica* were picked before sunrise. These leaves are chopped and then crushed and the dough is placed in a well-tied net and

everything is soaked in four litres of water. Fifteen to thirty minutes later, the net is pressed and the liquid obtained is mixed with 10ml of soap solution which is often used as a wetting agent. One hundred grams of *Allium sativum* pods are chopped and crushed and the paste was mixed with two teaspoons of vegetable oil, 10l of water and 10ml of soap solution. To evaluate the effect of biocides on soil nematofauna, plant extracts were used with a determined dose for a given area (Table 1).

**Table 1:** Dosage of biocides used

Biocides	Amount of material in the raw solution (Kg)	Volume of raw solution (litres)	Volume of raw solution used for treatment (litres)	Volume of water used for treatment (litres)	Surface to be treated (square metres)
<i>Azadirachta indica</i> -based	2	4	1	9	250
<i>Allium sativum</i> -based	0.1	10	10	10	1000

To facilitate the adhesion of biocides on leaves, a few drops of liquid soap were added.

**5. Phytosanitary treatments**

The okra plants were treated twice simultaneously with both aqueous extracts. The first treatment coincided with the vegetative phase of the okra and the last during the reproductive phase. The following dosages were applied: dilute 1l aqueous extract of *Azadirachta indica* leaves with 9l water to spray 250 square meters; dilute all the aqueous extract of *Allium sativum* pods with 10 litres of water per 1000 square meter.

**6. Soil sampling**

Soil samples were collected and labelled before sowing, during the flowering and fruiting phase in the middle of each plot using a transplanting trough to detect the presence or absence of phytoparasitic nematodes.

**7. Extraction of nematodes**

The extraction was carried out in laboratory by the tube method. The process is as follows:

- Approximately 200 g of soil is introduced into a tube with a capacity of 1500 ml;
- The tube is then filled with tap water;
- The tube content is mixed until a suspension is obtained and is let to settle for a moment; the soil thus settles at the bottom of the tube and nematodes, lighter, remain in suspension longer;
- With the help of a metal sieve whose meshes are 50 µm, the suspension of nematodes is filtered; these are thus retained at the bottom of the sieve and will then be recovered in a small beaker.
- The tube is again filled with tap water and the exercise is repeated three times.
- The beaker content is finally poured into a sieve covered with three layers of cellulose wadding (kleenex)

paper); the whole is placed in a Petri dish containing clear water (Baermann system). After 48 hours, the nematodes passed through the sieve and into the water in the petri dish.

A solution of nematodes is then obtained; the latter are then taken using a stereomicroscope for a photonic microscope study.

## 8. Nematodes treatments

It includes the fixation, counting, dehydration, impregnation and the mounting of nematodes on slides.

### 8.1 Fixation

The fixative used is a 4% formaldehyde solution neutralized (pH=7) by CaCO<sub>3</sub> powder. Nematodes are hot-fixed (about 60°C) and stored in tubes.

### 8.2 Nematodes counting

Depending on their size and habitus, nematodes are divided into groups and the number of specimens in each group is counted using a counting plate with a binocular stereomicroscope.

### 8.3 Dehydration

A few specimens of each type of nematode encountered during counting are picked up and transferred to saltcellars filled with fixative. Saltcellars, without a lid, are then placed in an alcohol (96% ethanol) desiccator, which is placed in a 40° C oven for at least five days. At this stage, the nematodes are in an ethanol-fixative predominantly ethanol mixture.

### 8.4 Impregnation

After dehydration, saltcellars are removed from the desiccator and the ethanol-fixative mixture is replaced by a glycerine alcohol solution consisting of 95% ethanol 96% and 5% glycerin. These saltcellars, with their lid leaving a small opening, are placed in the oven at 40° C until the alcohol evaporates completely after 24 to 48 hours. Nematodes, now in pure glycerine, can be mounted on slides.

### 8.5 Mounting on slides

The mounting is carried out under a stereoscopic microscope and according to the following procedure:

- A paraffin ring is deposited on a Cobb blade;
- A drop of glycerine is placed in the middle of the paraffin ring;
- Specimens are placed in a drop of glycerine;
- The preparation is covered with a lamella;
- The lamella is then heated until the paraffin ring liquefaction.

### 8.6 Observation and identification

On each specimen, the organization of the pharynx and especially that of the stylet was observed in order to determine whether it is a free nematode or a phytoparasitic nematode.

## 9. Data processing

Raw data was entered and filed in a Microsoft Excel 2013 spreadsheet that represented our database. The statistical analysis (non-parametric test of Kruskal Wallis) was done with R software version 3.5.2. Frequency of occurrence and

relative abundance were calculated using the following formulas.

Relative abundance:  $n_i/N$  With  $n_i$  = number of individuals in the same group and  $N$ = total number of individuals recorded.

**Frequency of occurrence:**  $V_i/V_t$ , with  $V_i$  = number of records where order is present and  $V_t$  = total number of records.

## Results

### 1. Composition of the soil nematofauna

The results of the nematofauna identification showed the presence of two trophic groups within the experimental plot: phytophagous and free nematodes. Among the phytophagous nematodes, there are those belonging to the Order of Rhabditida and those belonging to the Order of Dorylaimida (Table 2). This table shows a dominance of phytophagous nematodes with a representativeness rate of 53.6% in Rhabditida. However, a very low presence of Dorylaimida phytonematodes is noted.

**Table 2:** Distribution of nematofauna into groups

Groups	Phytoparasites nematodes		Free nematodes
	Rhabditida	Dorylaimida	
Number of nematodes	1050	4	904
Abundance (%)	53.6	0.2	46.2

### 2. Dynamics of phytoparasitic nematodes population

#### 2.1 Depending on the growth of the okra plants

The Kruskal-Wallis test rank of the R software, on the data concerning the effect of plant growth on the variation in the population of phytorhabtid nematodes appeared not significant (p-value = 0.17); the growth of okra plants did not have an effect on the phytorabstid population.

#### 2.2 Depending on applied treatments

The Kruskal-Wallis test is applied to data on the action of biocides used on nematodes. Results showed that biocides used had no effect on nematodes.

#### 2.3 Effect of biocides on okra production

The weight of fruit per parcel was weighed at each harvest. The total sum of elementary yields of each crop allowed us to have the final production of each plot (Table 3).

The average production per parcel is 27,758.9 grams, and the expected average production per parcel is 26,000 grams. The Kruskal-Wallis rank test, on the data concerning the effect of biocides on the yield of okra plots, appeared to be non-significant (p-value 0.49); so, there is no significant difference between the yields of treated plots and controls.

**Table 3:** Production, per plot, of okra

Np et Ta	Weight (gram)
P1 (Te)	16496
P2 (Ai)	19100
P3 (As)	22300
P4 (Ai)	26105
P5 (As)	36890
P6 (Te)	35369
P7 (As)	33130
P8 (Te)	31470
P9 (Ai)	28970
Total	249830

Since neither the application of biocides nor plant growth has an effect on the variation in nematode population, nor the expected yield per plot is achieved, it can be assumed that okra is resistant to the types of nematodes encountered.

### Discussion

The phytoparasitic nematodes inventory showed that the Rhabditida family is dominant in the site. This dominance of Rhabditida is also noted in the results of Touré (2012) [12] on the characterization of phytophagous nematodes in the Niayes area. Our study, which was carried out for the first time in Senegal on this speculation, also revealed a very low level of representativeness of phytodorylaimides. The low percentage of Dorylaimida phytonematodes noted could be due to the fact that the mesh of extraction sieves used is inappropriate for these types of nematodes which are relatively large (Faye, 2011) [3]. It also showed a low diversity of nematode species in okra, contrary to the study by Danso and Abugria (2021) [11] which showed a large number of nematodes on okra plants in Ghana. These authors identified six genera of nematodes in okra farming: *Meloidogyne*, *Pratylenchus*, *Xiphinema*, *Helicotylenchus*, *Rotylenchulus* and *Scutellonema*. The results obtained on the application of biocides showed no effect on the nematodes present in the culture despite the work of Hellpap (1995) [7] which showed that neem contains compounds such as nimbin, deacetylnimbin and thionemone, which have nematocidal effects and act by inhibiting egg production and hatching, as well as mobility and penetration of second-stage juveniles into plant roots. This could be due to the fact that treatments have always been followed by showers, or that nematodes are insensitive to the biocides used. Our results are different from (Haougui *et al.*, 2008) [6] who have demonstrated the effectiveness of neem and castor leaves on *Meloidogyne javanica* and recommended them as a substitute for synthetic nematicides that are toxic and often inaccessible to small producers.

There is no significant difference between the productions of treated boards and controls. Since neither the application of biocides nor the growth of the plant had no effect on the variation in the nematode population and the expected yield per plot is achieved, it can be assumed that okra is resistant to the types of nematodes encountered. Systematic research on nematode resistance has identified resistant lines within cultivated or related species (Roger Boerma and Richard, 1992) [12].

### Contribution/Originality

This article contributes to the search for solutions for crop nematodes control. It is interesting, since this is the first time such a study has been carried out in Senegal. On the other hand, it has used natural substances to control nematodes to avoid impacts on human health and the environment.

### Conclusion

Our study, which aimed to characterize the population of nematodes affiliated with the ISAE experimental site and evaluate the effects of aqueous extracts of *Allium sativum*, of *Azadirachta indica* and that of the growth of okra plants on the population dynamics of nematodes, has identified two groups of phytoparasitic nematodes: Rhabditida and Dorylaimida. In addition, the nematofauna encountered in this okra field is dominated by the presence of phytophagous nematodes belonging to the Rhabditida group

(53.6%). The okra is resistant to phytophage nematodes encountered on this site. In addition, the revision of nematode extraction techniques and the resumption of work during a dry season, to be able to control the dosage of biocides used, should be considered.

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