

Effect of the Extract of the Mixture of *Tagetes Patula* on the Development of the Nematode *Ditylenchus Dipsaci* on Broad Bean (*Vicia Faba*)

Saadi Inesse¹, Boumaraf Belkacem²

^{1,2} Laboratory of promoting innovation in agriculture in arid regions, University of Mohamed Kheider, Biskra, Algeria

Abstract

Stem and bulb nematode: *D. dipsaci* constitutes a fairly serious threat to beans in Algeria, particularly through its transmission by seed. the effect of aqueous extracts of some plants tested in vitro, the data obtained showed that all the extracts tested caused mortality of *D. dipsaci* and the mortality rate depends on the period of exposure, the concentration and the nature of the extract. In vivo, the application of *Tagetes patula* extracts to broad beans is more effective before but not after planting.

Keywords: *Ditylenchus Dipsaci*, *Vicia Faba*, Biological Control, Nematicide, *Tagetes Patula*, Phénamiphos

1. Introduction

Phytophagous nematodes, unlike the zooparasitic nematodes that have always been known, have gone unnoticed for a long time, and often still are, due to their microscopic size and the fact that they are always hidden in the soil or indoors. plant tissues. Ignorance of their presence has often been covered by the general term "soil tiredness".[1]

The phytosanitary problems caused by these pests have a very significant economic impact on a global scale, because they attack field crops as well as market gardening, flower and fruit crops. In Europe, they are responsible for damage reaching 10% of cereal production and lead to harvest reductions of 20 to 30% in Mediterranean citrus orchards.

The objective of our work is to show the interest of natural substances as an alternative means against the stem nematode: *Ditylenchus dipsaci*.

Our study focuses on the effect of extracts from some plants on the mortality of juveniles of *Ditylenchus dipsaci*, and the effectiveness of the aqueous extract of *Tagetes patula* on the development of the nematode on beans

2. Materials and methods :

1.1 Study on the different aqueous extracts on the mortality of *Ditylenchus dipsaci*

Origin of the plants studied

The plants tested during our experiment all come from the National Institute of Agronomy and belong to the families of:

- A. **Meliaceae: *Melia azedarach*:** This species is very widespread in tropical countries and was introduced into North Africa where it developed as an ornamental tree from the coast to the Oases. It is a tree that can reach fifteen meters in height whose pinnate leaves are composed of distinct leaflets. The flowers have pale purple or white petals, the fruits are spherical or ovoid. It is cultivated in several relatively temperate regions, for example in the north of North Africa where it is sometimes naturalized; all parts of the plant are purgatives, vermifuges, insecticides and nematicides. The bark of the root has been used against scrofula and leprosy .[2].
- B. **Verbenaceae: *Lantana camara*:** Lantana: It is a shrub 1 to 3 meters high, sometimes almost climbing, with simple, toothed and rough opposite or whorled leaves. The pubescent branches have short hooked thorns. The opposite leaves are oval, triangular at the top, and regularly toothed on the edge. The flowers vary in color from yellow to mauve, but there are many white and bright orange varieties [2].

The leaves have a high content of essence called lantanone and several terpenes, among which citral predominates. The essential oil is rich in sesquiterpenes, another compound called lantanine and lantadene (these are triterpene acids).

C. **Asteraceae: *Tagetes patula*** : Marigold. Ornamental plant native to Central America widely cultivated in Mediterranean countries. The stem is robust, 30cm high, bearing pinnate, glabrous leaves and large double flowers of bright yellow, orange-yellow or coppery brown. There are many varieties (dwarfs). Its cultivation is extremely easy and adapts to all poor and dry soils, in sunny or partial shade. It supports the maritime climate. This plant is very floriferous. It contains a certain number of active principles, the chemical structures of these substances extend very widely to various families of molecules, the main ones of which are biethinyl derivatives and alpha -terthienyl [3]. These plants are widely used in the medical, veterinary, pharmaceutical and cosmetic industries. They contain several constituents such as alkaloids, amino acids, essential oils. These compounds are known for their bactericidal, fungicidal, insecticidal and nematocidal activity [4].

Biological material

The strain of *Ditylenchus dipsaci* that we used during our experiment comes from infested bean plants from the Biskra region. Located in the South-Eastern region of Algeria. This region is the first agricultural region in the plasticulture sector.

Plant material

The variety of bean used for carrying out this test is the Aguadulce variety: It is a variety widely used by farmers, it is very sensitive to the nematode: *Ditylenchus dipsaci*.

Obtaining aqueous extracts from plants

The extraction of plants can be done using several methods including extraction from alcohols or water. We opted for the latter; it is recommended by several authors [5],[6]. The technique consists of grinding leaves, stems, roots or flowers in a blender containing distilled water or in certain cases mixing the different parts of the plants used in our experiment. The solutions obtained are centrifuged at 3000 revolutions for a few minutes, then sterilized by microfiltration ("Watman" 0.22 µm filter). The solutions thus obtained constitute the stock solutions called biological solutions from which the different dilutions are prepared (S/2,S/5).

Chemical treatment

The product used during our experiment is phenamiphos (Némacur). The choice of this product is

due to its frequent use by farmers and its availability on the market. The characteristics of this nematocide have already been presented in Chapter III.

5. Effect of foliar extracts on mortality of *Ditylenchus dipsaci*

3. Materials and method

This test makes it possible to highlight a possible nematocidal action in the literal sense of the word. The test was carried out in 50 mm diameter Petri dishes which contain the biological solution at different concentrations 100%, 50%, 25%. We deposit around a hundred (100) juveniles of *Ditylenchus dipsaci* aged between 24 hours and 48 hours. Counting of dead juveniles was carried out 24, 48 and 72 hours later. For each treatment, we performed 3 repetitions.

Distilled water and a solution of the nematocide Ethropophos with 10% active ingredient were taken as controls. The results are expressed as percentages of mortality according to the formula below, and the effectiveness of the different extracts is determined by the regression coefficient (Chatterjee et al., 1982).

-Mode for calculating the mortality percentage

$$\text{Mortality percentage \%} = \frac{\text{Number of dead nematodes}}{\text{Number of live nematodes}} \times 100$$

The test was carried out in plastic pots with a capacity of 1.5 kg filled with a mixture of soil and potting soil (2/3.1/3), previously sterilized.

1 Preparing seedlings

Sowing was carried out in trays containing soil previously sterilized after disinfection of the bean seeds in a sodium hypochlorite solution.

2. Transplanting

The Aguadulce variety bean seedlings with 2 to 5 leaves were transplanted at the rate of one seedling per pot for post-planting and chemical treatment. Regarding the preventive treatment, the transplanting was carried out ten days after the application of the treatment, then the pots were covered with plastic film.

3. Inoculation

A few days after transplanting the seedlings were inoculated with *Ditylenchus dipsaci* at a rate of 200 nematodes per pot.

4. Treatment

A few days after inoculation, five ml of the aqueous extract of *Tagetes patula* at concentrations (100% and 50%) were applied to each bean seedling.

The experimental device

The experimental design is a complete random block including the following treatments:

A. Preventive treatment:

- ✓ Treatment before planting at a concentration of 100%
- ✓ Treatment before planting at a concentration of 50%

B. Curative treatment:

- ✓ Treatment after planting at a concentration of 100%
- ✓ Treatment after planting at a concentration of 50%

C. The witnesses are represented by:

- ✓ A control: bean + nematodes
- ✓ A chemical treatment with Ethoprophos (Mocap) was applied after planting at a dose of 50 kg/ha.

5 repetitions were carried out for each treatment, for a total of 30 pots.

These pots were arranged in a complete random block in the conditions and watered and fertilized regularly

T1: Witness

T2: 100% treatment before planting

T3: 50% treatment before planting

T4: 100% treatment after planting

T5: Treatment 50% after planting

TC: Chemical treatment (Ethoprophos)

The parameters used to evaluate the effectiveness of the different biological solutions of *Tagetes patula* against *Ditylenchus dipsaci* are:

*the rating of symptoms according to the scale established by [7] ..

*recording the growth of bean plants by measurements five months after setting up the test.

* determination of the number of nematodes in the stems five months after setting up the test according to the incubation method described previously

*determination of the number of nematodes in seeds

*the effect of *Tagetes patula* extracts on the yield components .

4. Results and discussion

Effect of different aqueous extracts on the mortality of *Ditylenchus dipsaci* :

For each aqueous extract, the average mortality percentage is calculated. The mortality percentage in the control is zero; therefore, the results were not expressed as a corrected mortality percentage. However, we proceed to transform the results obtained during our test into probits.

From these values, it is possible to plot the regression lines using probits of mortality percentage as a function of the logarithms of the doses used.

The analysis of the results presented in table (1) shows the aqueous extracts both foliar and root as well as the mixture of different parts of *Tagetes patula* and the foliar extracts of *Lantana camara* (fig 6), and those of *Melia azedarach* (leaves and seeds) exhibit nematocidal activity against juveniles of *Ditylenchus dipsaci* ; in fact the mortality percentage increases when the concentration and the period of exposure increase.

Thus, at a higher concentration S (100%) of the leaf extracts and at an exposure period of 72 hours, the percentage of mortality of the extracts obtained from leaves of *Lantana camara* and the mixture of the different parts *Tagetes patula* (fig 1), and seeds of *Melia azedarach* , cause the highest mortality rate and reach 82, 80 and 78% respectively.

At this same concentration and an exposure period of 24 hours, the mortality rate is 52, 54 and 61% respectively for the leaf and root extract and the mixture of *T patula* (fig 2 and 3) and and it is 55, 58 and 58% for the leaf extract of *Melia azedatrach*, *Lantana camara* and *M azedarach* (seed).

At an average dose S/2 (50%) and after 72 hours of treatment, these percentages are 65% for the mixed extract of *T. patula* and 63%, 58%, and 72% respectively for the extracts of *L. camara*, *M azedatrach* (leaves) and *M. azedarach* (seeds).

At low concentrations (20%), All the extracts tested show significant mortality of juveniles (L4), except that of *T. patula* (leaves).

After 24 hours of exposure to concentrations of 20%, all the leaf extracts tested showed a mortality percentage of less than 50%.

The comparative study of extracts from different parts of *Tagetes patula* showed that root extracts are more effective than foliar extracts and reach 52 and 65% respectively for exposure periods of 24 and 72 hours.

Chemical treatment causes mortality of juveniles (L4) of 72% respectively for an exposure period of 24, 48 and 72 hours. Finally, the juveniles remained active in the control (distilled water) during the three exposure periods; the majority of the nematodes remained alive. The effectiveness of the different aqueous extracts was also evaluated by regression lines.

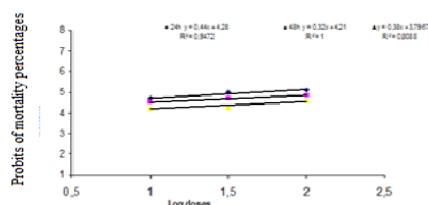


Fig 1 Regression line of probits at different doses used in the aqueous extract of *Tagetes patula* (leaves) on *Ditylenchus dipsaci*

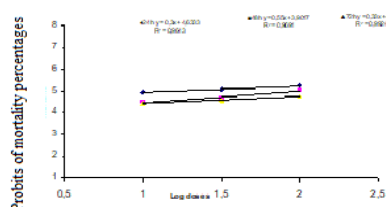


Fig 2 Regression line of probits at different doses used in the aqueous extract of *Tagetes patula* (roots) on *Ditylenchus dipsaci*

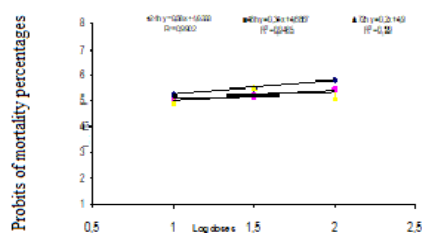


Fig 3 Regression line of probits at different doses used in the aqueous extract of *Tagetes patula* (mixture) on *Ditylenchus dipsaci*

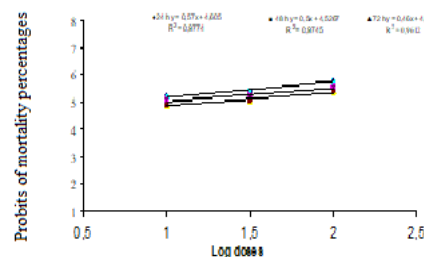


Fig 4 Regression line of probits at different doses used in the aqueous extract of *Melia azedarach* (seeds) on *Ditylenchus dipsaci*

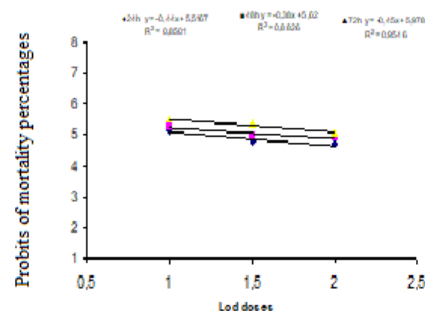


Fig 5 Regression line of probits at different doses used in the aqueous extract of *Melia azedarach* (Leaves) on *Ditylenchus dipsaci*

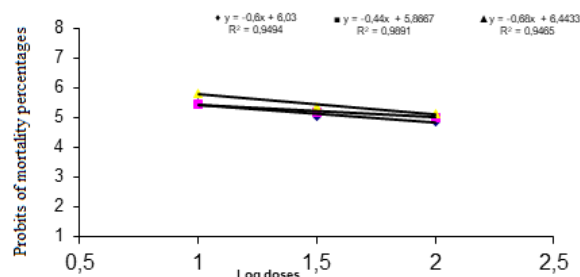


Fig.6: Regression line of probits at different doses used in the aqueous extract of *Lantana camara* (leaves) on *Ditylenchus dipsaci*

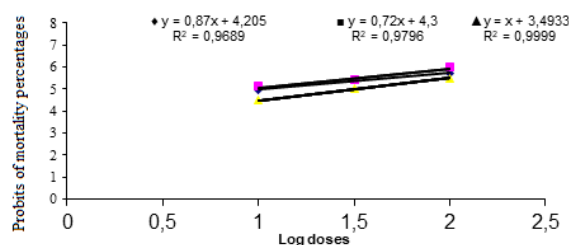


Fig 7: Regression line of probits at different doses of Nematicure on *Ditylenchus dipsaci*

Table 1: Effect of aqueous extracts of some plants on *D. mortalityitylenchus dipsaci*

Each value represents the average of three repetitions

Plants tested and exposure period	Mortality percentages			Probits		
	S (100%)	S/2(50%)	S/5 (20%)	S (100%)	S/2 (50%)	S/5(20%)
<i>Tagetes patula</i> (leaves)						
24	52	42	25	4.69	4.53	4.23
48	58	46	34	5	4.69	4.16
72	63	52	42	5.13	4.85	4.69
<i>Tagetes patula</i> (roots)						
24	54	48	38	4.95	4.48	4.42
48	59	54	42	5.05	4.67	4.56
72	65	56	52	5.25	5.03	4.75
<i>Tagetes patula</i> (mixture)						
24	61	52	48	5.23	5.05	4.95
48	73	56	50	5.47	5.15	4.19
72	80	65	56	5.81	5.39	5.15
<i>Lantana camara</i> (leaves)						
24	58	52	45	5.47	5.05	4.87
48	67	56	50	5.44	5.18	5
72	82	63	55	5.81	5.33	5.13
<i>Melia azedarach</i> (leaves)						
24	55	40	38	5.13	4.75	4.69
48	62	49	46	5.28	4.97	4.90
72	69	58	52	5.50	5.36	5.05
<i>Melia azedarach</i> (seeds)						
24	58	52	46	5.20	5.05	4.90
48	66	59	52	5.41	5.23	5.05
72	78	72	64	5.77	5.55	5.36
Chemical treatment (Némacur)						
24						
48	0	0	55	0	0	0
72	0	0	66	0	0	0
	0	0	72	0	0	0
Witness						
24	0		0	0	0	0
48	0		0	0	0	0
72			0	0	0	0

I.2. Effect of the aqueous extract of the *Tagetes patula* mixture on the development of *Ditylenchus dipsaci* on broad beans.

The nematological analysis of the bean stems from the different treatments allowed us to determine the average number of nematodes in the stems, these results are recorded in table (2).

These data allow us to note the following points:

- Before planting the average number of nematodes is respectively 686.4 and 495.6 per plant for concentrations of 50 to 100%.
- After planting this number reached 889 and 747.8 nematodes per plant respectively for the 100% and 50% concentrations of the *Tagetes patula* extract.

- In the case of chemical treatment, the average number of nematodes recorded is 703 nematodes per plant. - The highest numbers of *D. dipsaci* were recorded in the control with an average of 1438.2 nematodes per plant.

- Finally, the table shows us that the preventive treatment at concentrations 50 and 100% is the most effective with a percentage reduction of 52 and 65% respectively.

- The chemical treatment revealed an effectiveness similar to that of the preventive treatment at 50%

- After planting at concentrations 50 and 100%, the percentage of nematode reduction is lower and is 38 and 48% respectively.

The analysis of variance based on the Fisher test shows that there is a very highly significant difference

between the different treatments compared to the control (Table: 6).

Table 2: Effect of different treatments of the foliar extract (mixture) of *T.patula* on the numbers of *Ditylenchus dipsaci* on broad beans.

Treatments	Average number of nematodes per bean plant	Reproduction Factor $R = P_f/P_i$	Percentage decrease (%)
Before planting			
100% treatment	495.6	2.4	65.5
Treatment 50%	686.4	3.43	52.28
After planting			
100% treatment	747, 8	3.73	48
Treatment 50%	889	4.44	38.19
Chemical treatment	703	3.5	51.12
Witness	1438, 2	7.19	-

P_f = final population, P_i = initial population

Table 3: Ranking of homogeneous groups of the average numbers of *D. dipsaci* per plant of the different treatments compared to the control according to the Fisher Test (LSD)

Treatments	Average	Homogeneous groups
treatment before planting	495.6	D
treatment after planting	889	B
treatment before planting	686.4	VS
Treatment 50% after planting	747.8	VS
Witness	1438.2	HAS

In fact, the classification of the average numbers of *D. dipsaci* per plant tells us that there are 4 groups. However, treatment before planting at 100% and 50% concentrations and after planting at concentration of 50% present a non-significant difference in contribution to chemical treatment (table: 4);

Table 4: Classification of homogeneous groups of the average numbers of *D. dipsaci* per plant of the different treatments compared to the chemical treatment according to the Fisher Test (LSD)

Treatments	Average	Groups homogeneous
treatment before planting	2.4	B
Chemical treatment	3.5	VS
treatment after planting	3.73	B
treatment before planting	3.43	B

Treatment 50% after planting	4.44	HAS
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The results presented in the table show that the reproduction factor is high in the control and reached 7.19, the lowest was noted during the pre-planting treatment with the biological solution of *T. patula* at a concentration of 100%.

The treatments before planting at 100% concentration and the chemical treatment have a similar reproduction factor with 3, 43 and 3.5 respectively. We can say that these results show that the nematode multiplied well, in fact the conditions of the experiment were favorable to the development of the disease.

1.3 The effect of *T. patula* extract on the severity of the disease:

The severity rating according to the scale of [7] was assigned throughout the trial to determine the period of symptom onset.

The first symptoms of the disease due to *D. dipsaci* were observed 28 days after inoculation on untreated plants (control), the disease was manifested by the presence of necrosis at the crown, this necrosis progressed gradually at the level of the stems. Symptoms of swelling were noted after the 36th^{day}.

The manifestation of symptoms worsened at the end of the trial. Thus, stem deformations and internode shortening were observed.

Virtually similar symptoms were noted in the treatment after planting at a concentration of 50% and the control with more accentuated symptoms. As a result, the average severity for these two treatments is 8, 2 and 7.4 respectively.

Table 5: Effect of different treatments on the average severity of the disease

The different treatments	Severity of the disease according to the scale of Hanounik et al., (1986)
Before planting	
100% treatment	4.2
50% treatment	5.4
After planting	
100% treatment	6.2
50% treatment	7.4
Chemical treatment	
Witness	5.8
	8.2

I.4 I. Effect of the aqueous extract of the mixture of *T. patula* on plant growth:

The effectiveness of the *T. patula* extract was also estimated on the growth of bean plants. The results are shown in figure (3). The average values of the heights of the bean plants are greater at the level of the chemical treatment and that of the treatment before planting at the 100% concentration; in fact they are respectively 76.6 and 72.6 cm.

Regarding the treatments before planting at 50%, and after planting at 100%, the values obtained are significantly similar to that of the control with respectively 64.2, 63.6 and 62.6 cm. The percentage increase in the growth of bean plants is significant except for and the treatments before planting at 100% concentration (Fig 3) and chemical is reached is respectively 15.97% and 22.36.

The treatments at 50% before and after planting and before planting at 100%, the growth percentage is negligible and varies between 0.3 to 2.5%.

Indeed, the analysis of variance based on the Fisher test of plant height (in cm) reveals a significant difference for these two treatments. However, for the other treatments the difference is not significant compared to the control (Table: 6). *Tagetes patula* on the number of nematodes in seeds and the effect on yield components could not be studied, due to attacks by diseases such as *Botrytis*, *fusarium* and pests. such as aphids and sitones which appeared at the end of the culture, therefore, it would be desirable to continue this work by studying these parameters.

Table 6: Classification of homogeneous groups according to the average height of the plants compared to the control

Treatments	Averages	Homogeneous groups
treatment before planting	72.6	HAS
treatment after planting	62.8	B
treatment before planting	64.2	B
Treatment 50% after planting	63.6	B
Witness	62.6	B

Comparison of different treatments compared to chemical treatment by the test Fisher's revealed the presence of two homogeneous groups in table (7).

Table 7: Classification of homogeneous groups, function of average plant height in relation to chemical treatments

Treatments	Averages	Homogeneous groups
Chemical treatment	76.6	HAS
treatment before planting	72.6	HAS
treatment after planting	62.8	B
treatment before planting	64.2	B
Treatment 50% after planting	63.6	B

5. Conclusion

This study allowed us to demonstrate the *in vitro* effectiveness of the different extracts in the three plants tested on the mortality of *Ditylenchus dipsaci*.

Plants are an important source from which biopesticides can be obtained. Insecticides of plant origin such as pyrethrum have been developed and marketed against insects, and compounds with nematocidal activity have been isolated from plants, particularly those of the *Asteraceae* and *Meliaceae* families.

The nematocidal activity demonstrated during our tests can be attributed to the compounds present in the leaves, seeds, or roots of the plants tested. Indeed, these plants are very toxic and very rich in essential oil, the latter contains around ten compounds including terpenes and alkaloids .

Thus, derivatives of 2-bithienyl, and compounds such as 2,3,4,2-thienyls and several methyl polythienyls have been isolated from *Tagetes erecta* and *T. patula* and have proven to be effective and exhibit strong nematicidal activity ([8]; [4].

Other compounds characterized as thiophenes have also been identified in different species of *Tagetes* ([9] [4]. The presence of biocidal metabolites known as thiophenes are also present in the leaves and roots of *T. mendocina* and *T. argentina* [10], the latter plants being recognized as having nematicidal activity.

Finally, compounds belonging to the flavonoid group have been identified in the genus *Tagetes*. These constituents also have nematicidal activity against many phytophagous nematodes ([4], [11].

Similarly, compounds have been isolated respectively from the leaves of *Lantana camara* identified as lantonoside, lantanone and camarinic acid. Regarding the major compounds identified in the leaves and seeds of *Melia azedarach* are the limonides such as azadirachtin, nimbin and salamin (groups of triterpenes) are the best known. Steroid and glycoside fractions were also determined. All these compounds exhibit very strong nematicidal activity against numerous phytophagous nematodes [12], [13] in: [4].

Other chemical compounds are present in the leaves such as tannins, estragol, lineol, phenols, tannins, and saponins, in fact, the latter constituents are known for their intervention in the defense mechanisms of plants against nematodes.

The mechanisms of action of these substances still remain very little known; some authors hypothesize that the relative sensitivity of different groups of nematodes to chemical compounds contained in plants is a function of the permeability of the cuticle; in fact the molecules cannot have access to the tissues of plant-parasitic nematodes and penetrate through the cuticle; however other authors argue that the mode of action is similar to that of insects, that is to say that these molecules act at the level of the nervous system by inhibition of acetylcholinesterase [14] and [15].

the results concerning the incidence of the extracts on the development of the disease which revealed a reduction in the numbers of *D. dipsaci* and a reproduction rate of 4.2 to 7.19 can be attributed to the toxic action of the biological solutions which contain nematicidal toxins, diffused in the soil could neutralize or even disorient the nematodes. Other authors think

that these, by dispersing in the soil, kill the nematodes [16].

According to [17] the externalization of symptoms is related to the reproduction factor. According to the same author, the final populations of *D. dipsaci* vary greatly and can reach tens of thousands of individuals when conditions are favorable.

Furthermore, the method of application of the extract before planting is the one which induced a significant reduction in the numbers of *Ditylenchus dipsaci*; on the other hand, the treatments after planting recorded a lesser reduction, therefore it would be desirable to carry out tests by carrying out two or three applications of the plant extract after planting.

The effect of plant extracts has been the subject of numerous studies with regard to several phytophagous nematodes. Thus, the effectiveness of *Tagetes erecta*, *Origanum vulgare*, *Origanum syriacum* (Lamiaceae), *Peganum harmala* (Zygophyllaceae), *Ceratonia siliqua* (Fabaceae) has been reported against *Meloidogyne*.

[18] and [19] reports the effectiveness of *Ruta graveolens* (Rutaceae), *Aristotelia chilensis* (Elaeocarpaceae) and *Cestrum parqui* (Solanaceae) against *Ditylenchus dipsaci*.

Finally, [20] report the effectiveness of *Ruta graveolens* against *D. dipsaci* at low concentrations for a period of 24 hours. These same authors also record the nematicidal activity of aqueous extracts of *Asparagus* spp and *Alium sativa* (Liliaceae), *Tagetes* spp against juveniles of *Ditylenchus dipsaci*.

Given the nematicidal potential of *Tagetes*, it would be desirable to continue this work, particularly by determining the mechanisms involved and identifying the compounds responsible for this action.

Finally, test this plant in natural conditions as a catch crop with the bean in order to determine its method of application (density, period, etc.).

Given current concerns, the use of nematicidal plants can constitute a very interesting and promising future path for Algerian agriculture.

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