Short Research Article

Pollen viability and fruit yield of coriander genotypes inoculated with different concentrations inoculation densities of *Meloidogyne incognita* race 1

ABSTRACT

Coriander is among the most consumed leafy vegetables in Brazil, employing a large number of people in its production chain. Among the limiting factors to the production of this horticulture, there is the nematode of the genus *Meloidogyne* causing by root galls nematodes. The present work was carried out aiming to at understanding the impact on coriander plants submitted to infected by the parasitism of *M. incognita* race 1. The number of galls were verified at 30 days after nematode inoculation as well as the plant survival up to the reproductive harvest stage. In addition, pollen viability and fruit yield in coriander plants of two cultivars (Verdão and HTV Dom Luiz), inoculated at sowing with six inoculum concentrations densities (0, 1,000, 2,000, 4,000, 8,000 and 16,000 eggs / cell plant) and evaluated in at a randomized complete block design with four replications were also analyzed. The plot was composed of one plant. The presence of the pathogen did not influence the pollen viability by means of the acetic Carmine and Alexander dyes. However, neither tetrazolium nor *in vitro* pollen germination means were efficient in the viability identification. Concentrations Densities of 8,000 and 16,000 eggs/cell plant did not allow the development of plants, leading them to death. Inoculation at sowing, and evaluation of the number of galls at 30 days did not limit the reestablishment of the plant development and fruit yield, up to the concentration density of 4,000 eggs/cell plant.

Keywords: Coriandrum sativum L., inoculum levels, Meloidogyne, Dyestuff.

1. INTRODUCTION

Gall nematodes (*Meloidogyne* spp.) are widely distributed as phytopathogens in tropical, subtropical and temperate regions of the planet. These worms are classified as one of the five main groups of economically destructive pathogens that affect world food production [1]. Within the phytonematological specialty, the *Meloidogyne* species constitute the most economically relevant group, due to their high degree of polyphagy and wide geographic distribution, being constant threats to the rural producers. The species with the most prominence within this genus for presenting themselves as the most cosmopolitan, polyphagous, and weedy to agriculture are: *M. arenaria, M. hapla, M. incognita* and *M. javanica*. The last two species are the most important from an economic point of view [2].

The species belonging to the genus *Meloidogyne* are obligatory endoparasites and the second stage juveniles (J2) are infectants, which penetrate the root elongation zone and migrate intercellularly until they reach the differentiating vascular zone, where they choose from 5 to 7 cells and insert their stylets injecting secretions to induce hypertrophy and hyperplasia forming giant cells, the galls and establishing the feeding sites. From that stage on, the pathogen starts to extract nutrients and photoassimilates from nearby tissues, mainly xylem and phloem [3]. Consequently, they promote, besides gall formation, leaf

UNDER PEER REVIEW

yellowing, defoliation, retarded growth and wilting as a symptoms, which collectively reduce plant vigor and cause mass loss and quality. It is estimated that 12.3% of the annual losses in agricultural production is due to the attack of gall nematodes, causing an economic loss of around US $ 157 billions [1].

In addition to underdevelopment, the formation of coenocytes, induced by gall nematodes, acts as a metabolic drain resulting in physiological imbalances, that is, a lack of macro and/or micronutrients [2]. Boron is a micronutrient that plays an important role in the growth of the pollen tube [4], and the lack of this element is a factor that can reduce pollen viability. In this context, plants were parasitized with gall nematodes, due to the presence of physiological disturbances, may undergo influence on the viability of the pollen grains resulting in reduced productivity. To verify pollen viability, various techniques can be adopted, which can be grouped into methods of direct *in vitro* determination [5]; direct *in vivo* determination [6]; and indirect methods such as staining from the biochemical reaction [7].

Coriander is among the various cultivated species attacked by gall nematodes. There is no cultivar in Brazil that is indicated as resistant to such pathogens, being necessary the search for superior genotypes. According to [8], the development of breeding programs aimed at providing resistance to phytonematodes is a process that requires adequate resources and methodologies. Currently, evaluation of the reaction of coriander genotypes to gall nematodes consists of sowing, inoculation at 15 days after sowing and evaluation at 45 days of inoculation, by destructive method for the extraction of pathogen eggs aiming at estimating the reproduction factor (RF) [9, 10, 8, 11].

The use of a methodology that does not promote the destruction of the plant, allowing the selection among and within segregating populations in the same selective cycle, may promote the attainment of promising results. Thus, the present work aims to at verifying the development of plants of two coriander cultivars inoculated at sowing with different concentrations densities of *M. incognita* race 1 inoculum and transplanted after 30 days, evaluating plant survival, percentage of viable and germinated *in vitro* pollen, and fruit yield.

2. MATERIALS AND METHODS

The experiment was conducted under greenhouse conditions, in the Department of Agronomy of the Federal Rural University of Pernambuco (UFRPE), located at 8°54'47"S, 34°54'47"W, 6m high, from July to October 2017. The monthly average temperatures recorded by the weather station of Recife Curado (automatic) varied in average between 22.9 - 28.6°C, for the minimum and maximum temperature, respectively [12]. The design was of randomized blocks in a factorial scheme of 2 (cultivars) X 6 (Nematode inoculum concentrations densities) with four replications, whose plot was composed by planted with one plant.

The reactions of the cultivars Verdão and HTV Dom Luiz against *M.incognita* were evaluated and handled in six concentrations densities (0, 1,000, 2,000, 4,000, 8,000 and 16,000) of *M. incognita* race 1. Sowing was carried out in expanded 128-cell polystyrene trays containing commercial substrate. The trays were irrigated, followed by nematode inoculation according to each treatment. Each block contained a plot with Santa Clara tomato cultivar (*Solanum lycopersicum* L.), with a susceptibility standard to gall nematodes, in order to verify the efficiency of the inoculum used.

UNDER PEER REVIEW

The inoculum was obtained from sources kept reared in tomatoes, Santa Clara cultivar, using the [13] and modified by [14] for the extraction of eggs of *M. incognita* race 1.(What time of egg inoculation?)

Irrigation was performed according to the water requirement of the crop, without drainage to prevent egg leaching. After germination, fertigation with nutrient solution containing macro and micronutrients was carried out three times a week, adapted from the proposal by [15], taking the same care not to drain and prevent consequent inoculum loss.

After germination, thinning was carried out leaving only one plant per cell. After 30 days of sowing and nematode inoculation, the roots of each plant of the plot were carefully washed in still water, to remove the substrate without damaging the root system, quantifying the number of galls in the root system and transplanting the seedlings (Figure 1) into 2L pots, properly identified (cultivar and inoculated concentration density), containing a substrate based on soil and humus mixture in a ratio of 3:1. These procedures were carried out at around 4:30 p.m., in order to favor seedling adaptation. Irrigation was carried out shortly after transplantation.

In the pots, the plants were irrigated daily according to the water requirement, and fertigation was applied three times a week. The survival of the plants was observed until the fruits were harvested.

At the beginning of flowering, when all the plants were flowering, flowers were collected to quantify the viable pollens in each cultivar as a result of the concentration density of the pathogen, seeking to verify if the presence and quantity of the pathogen influenced pollen viability.

The pollen was stained with acetic carmine [16], Alexander's solution [17] and tetrazolium salt solutions at concentrations of 0.25%; 0.50%; 0.75% and 1.0% [18]. Each dye reacts with a certain compound and/or structure of the pollen grain, promoting or not promoting grain staining. The stained grains are those considered viable. Acetic carmine indicates chromosomal integrity; Alexander solution contains acid fuchsin and green malachite which react with pollen wall protoplasm and cellulose [19], respectively, whereas tetrazolium salt provides an indication of the metabolic activity of the pollen grain , allowing the estimation of its viability [20] through the reaction of salt with hydrogen resulting from cellular respiration with red pollen [21] indicating the presence of functional enzymes such as peroxidase, esterase and dehydrogenase [22].

The flowers were collected after the anthesis from 7:00 a.m. to 9:00 a.m., stored in identified paper bags, and taken to the floriculture laboratory where the slides were prepared for visualization and counting of viable pollens in as result of the dye. For each dye, four slides per treatment (cultivar X inoculum) were prepared. The pollen of one flower was placed with the aid of a brush on the slide, and then two drops of dye were added before placement of the cover slip. The pollen was observed after 10 minutes of dye addition [19] under a 40X magnification microscope.

To evaluate the tetrazolium solutions, prepared by diluting the salt in distilled water, four flowers of each treatment were collected for each tetrazolium solution. With a pair of tweezers, the stamens of each flower were placed into an eppendorf, duly identified, containing 1 mL of a certain tetrazolium solution concentrations (0.25%, 0.50%; 0.75% and 1%). After that, the tubes were agitated for 20 seconds, so that the pollens had contact with the solution. The tubes were then wrapped with aluminum foil and kept at 25^{0}C for 24 hours in a BOD incubator [23, 18]. After this period, the solution of each tube was collected

UNDER PEER REVIEW

individually with a Pasteur pipette, placed onto a slide, and visualized in a 40X magnification microscope.

Proposed media were used for pollen germination *in vitro* for eggplant, citrus and some adaptations because no medium was found for coriander or other species of the Apiaceae family. For all media, phytagel was used instead of agar, leaving the medium more translucent, favoring pollen visualization.

Table 1. Media used aiming to at germinatng the coriander *in vitro* pollen grains

Medium	Reagents						Reference
	$Ca(NO_3)_2$	H_3BO_3	Sacarose	Phytagel	$MgSO_4$	KNO_3	
A	800 mg/L	200 mg/L	100 g/L	10 g/L	-	-	Salles et al., 2006
B	500 mg/L	120 mg/L	100 g/L	10 g/L	120 mg/L	100 mg/L	Tatis et al., 2013
C	4 g/L	3 g/L	10 g/L	10 g/L	-	-	-
D	4 g/L	3 g/L	10 g/L	-	-	-	-
E	-	40 g/L	200 g/L	10 g/L	-	-	-
F	-	40 g/L	100 g/L	10 g/L	-	-	-
G	20 g/L	40 g/L	200 g/L	10 g/L	-	-	-
H	20 g/L	40 g/L	200 g/L	-	-	-	-
I	20 g/L	40 g/L	400 g/L	-	-	-	-
J	20 g/L	20 g/L	400 g/L	10 g/L	-	-	-
L	10 g/L	5 g/L	50 g/L	10 g/L	-	-	-
M	10 g/L	5 g/L	10 g/L	10 g/L	-	-	-
N	5 g/L	5 g/L	10 g/L	10 g/L	-	-	-

For both dyes and in vivo media, 150 pollens per slide were evaluated, constituting the experimental unit.

After flowering and fruit filling phase, when they were dry, all fruits of each plant were harvested individually and stored in identified paper bags. Then, the number of fruits of each experimental plot was quantified, and 100 fruits of each plot were weighed on a precision scale.

The number of galls in the root system, number of fruits and weight of 100 fruits variables were transformed by $\sqrt{x}$ to meet the assumptions of the analysis of variance, and then submitted to ANAVA and Scott-Knott grouping test at 5% probability. Regression analyzes were performed for the decompositions within inoculum concentrations densities. The analyses were performed using the statistical software SISVAR [24], and the regression graphs were significant at 1% or 5%, elaborated in Excel.

3. RESULTS AND DISCUSSION

UNDER PEER REVIEW

In the treatments containing 16,000 eggs/plant In each cell, the plants died within a few days after germination. As for the treatment with 8,000 eggs /plant in each cell, the plants survived until the transplantation, but they were not able to complete the life cycle, due to the underdevelopment of both the root system and the shoot (Figure 1).

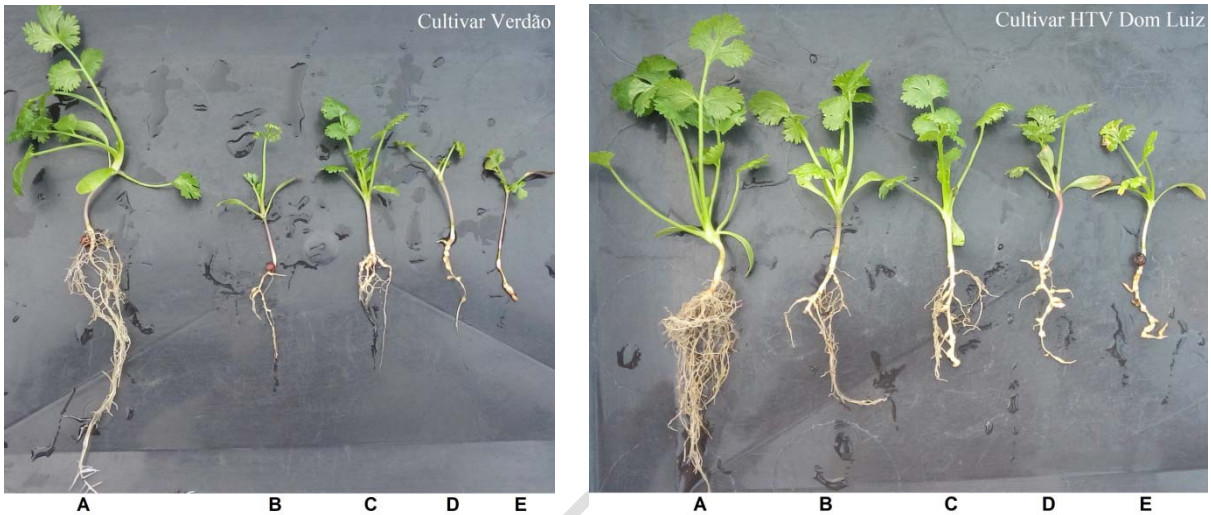


Figure 1. Coriander plants of Verdão and HTV Dom Luiz, 30 days after inoculation with different inoculum concentrations of *M. incognita* race 1. A) 0 eggs/plant; B) 1,000 eggs/plant; C) 2,000 eggs/plant; D) 4,000 eggs/plant; E) 8,000 eggs/plant.

It is possible to observe that with the increase of the concentration density of the inoculum, there is a reduction in the development of the root system and the shoot, simultaneously. A similar fact was observed by [25] in beet cultivars where a linear reduction of vegetative and root characteristics occurred as the inoculum level of *Meloidogyne incognita*, *M. javanica,* and *M. enterolobii* increased.

For the variables, number of galls in the root system, number of fruits and fruit weight, interactions between variation sources of variation and concentrations densities of inoculum X cultivars were significant at 1% probability. As for the variable, number of plants, there were significant differences only for concentrations density of inoculum at 1% of significance. The coefficients of experimental variation ranged from 12.98% (NGRS) to 27.08% (NF) (Table 2).

UNDER PEER

Table 2. Summary of the variance analysis of the number of galls in the root system (NGRS), number of plants (NP), number of fruits (NF) and weight of 100 fruits (WF) in two cultivars of coriander inoculated with six concentrations densities of inoculum of *Meloidogyne incognita* race 1.

SV	DF	MS			
		NGRS^{+}	NP	NF^{+}	WF^{+}
Blocks	3	0.09	0.02	2.02	0.002

Concentrations Inoculums	5	16.29**	2.02**	222.58**	2.79**
Cultivars	1	4.51**	0.02^{ns}	330.44**	0.001^{ns}
Inoculums*Cultivars	5	0.70**	0.02^{ns}	45.96**	0.05**
Error	33	0.07	0.02	3.02	0.01
CV%		12.98	22.35	27.08	13.72
Mean		2.09	0.65	6.41	0.75

$^{+}$ Data transformed by $\sqrt{x}$
** Significant at 1% probability
^{ns} Not significant

For the variable, NGRS, there was no significant difference between the cultivars only in the concentrations densities of 1,000 eggs/plant and 16,000 eggs/plant. InAt all other concentrations densities, the HTV Dom Luiz cultivar presented the highest number of galls compared to the cultivar Verdão (Table 3).

Table 3. Scott-Knott grouping test of the number of galls in the root system (NGRS), number of plants (NP), number of fruits (NF) and weight of 100 fruits (WF) in two coriander cultivars inoculated with six concentrations densities of *Meloidogyne incognita* race 1 inoculum, cultivated in greenhouse.

Variables	Concentration Density	Cultivars	
		Verdão	HTV Dom Luiz
NGRS	0	-	-
	1,000	10.50 a	10.75 a
	2,000	10.75 a	14.00 b
	4,000	7.00 a	10.75 b
	8,000	2.50 a	6.75 b
	16,000	0.00 a	0.00 a
NP	0	1.00 a	1.00 a
	1,000	1.00 a	1.00 a
	2,000	1.00 a	1.00 a
	4,000	0.75 a	1.00 b
	8,000	0.00 a	0.00 a
	16,000	0.00 a	0.00 a
NF	0	187.25 b	68.25 a
	1,000	259.75 b	67.25 a
	2,000	129.25 b	31.25 a
	4,000	199.00 b	63.75 a

UNDER PEER REVIEW

	8,000	0.00 a	0.00 a
	16,000	0.00 a	0.00 a
WF (grams)	0	1.37 a	1.13 a
	1,000	1.36 a	1.11 a
	2,000	1.05 a	1.05 a
	4,000	1.52 b	1.01 a
	8,000	0.00 a	0.00 a
	16.000	0.00 a	0.00 a

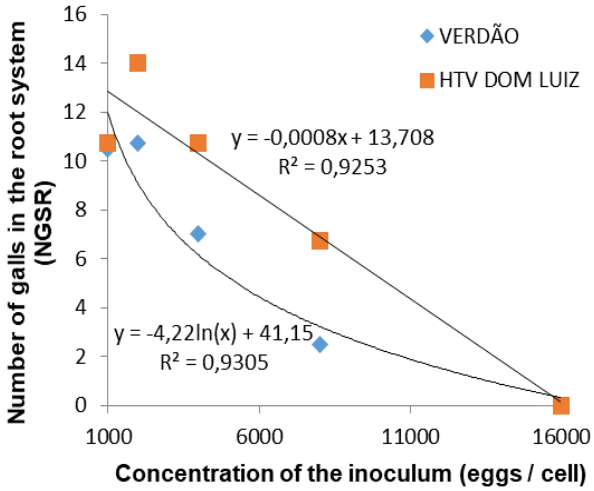
Means followed by the same lower case letter in the row did not differ statistically by the Scott-Knott test at 5% probability.

In the number of plants that completed the life cycle, in both cultivars, the concentrations inoculums of 8,000 and 16,000 eggs/plant did not allow plant survival, thus obtaining the lowest averages for this variable. For the remaining concentrations inoculums, there was no variation within or between cultivars, except in 4,000 eggs/plant where the cultivar Verdão obtained showed a lower average than the cultivar HTV Dom Luiz. In addition, the average was lower than those obtained for the concentrations inoculums of 0, 1,000, and 2,000 eggs/plant. Regarding the number of fruits, in the concentrations inoculums of 0 - 4,000 eggs/plant, Verdão cultivar had a higher number of fruits in relation to HTV Dom Luiz (Table 3).

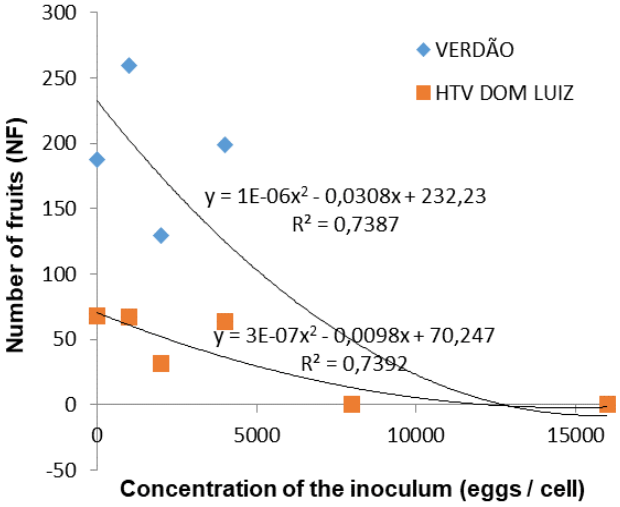
At the inoculum concentrations inoculums of 1,000 and 2,000 eggs/plant, both cultivars obtained the largest number of plants that completed the life cycle, allowing the evaluation of the number of galls in the root system with subsequent transplantation of the individuals selected for recombination and attainment of the new improved population.

Based on the obtained regressions, for the variable NGRS, it is noticed that with the increase of the inoculum concentration density, there is a reduction of the number of galls in both cultivars, fact justified by the underdevelopment of the plants under high concentrations densities of the nematode. As for the number of fruits, the cultivar Verdão was higher in the concentration inoculum of 1,000 eggs/plant, while with Dom Luiz HTV, the highest yields of fruits were obtained in concentrations inoculums of 0, 1,000 and 4,000 eggs/plant. In both cultivars, there was no fruit yield in the concentrations inoculums of 8,000 and 16,000 eggs/plant, for the plants that did not survive until the reproductive phase. By means of the obtained results, it is observed that the concentration inoculum of 1,000 eggs/plant allows a greater number of fruits for both cultivars (Figure 2).

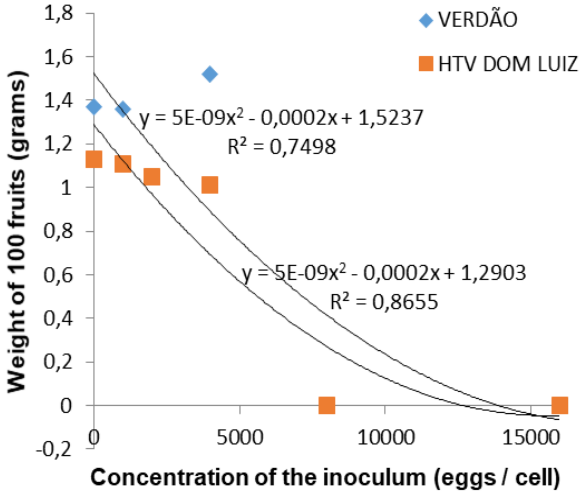
UNDER PEER REVIEW


VERDÃO
HTV DOM LUIZ
y = -0,0008x + 13,708
R² = 0,9253
y = -4,22ln(x) + 41,15
R² = 0,9305
Number of galls in the root system (NGSR)
Concentration of the inoculum (eggs / cell)
16
14
12
10
8
6
4
2
0
1000
6000
11000
16000

234


VERDÃO
HTV DOM LUIZ
y = 1E-06x² - 0,0308x + 232,23
R² = 0,7387
y = 3E-07x² - 0,0098x + 70,247
R² = 0,7392
Number of fruits (NF)
Concentration of the inoculum (eggs / cell)
300
250
200
150
100
50
0
-50
0
5000
10000
15000

235


VERDÃO
HTV DOM LUIZ
y = 5E-09x² - 0,0002x + 1,5237
R² = 0,7498
y = 5E-09x² - 0,0002x + 1,2903
R² = 0,8655
Weight of 100 fruits (grams)
Concentration of the inoculum (eggs / cell)
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
-0,2
0
5000
10000
15000

236

Figure 2. Number of galls in the root system (NGRS), number of fruits (NF) and fruit weight of Verdão and HTV Dom Luiz cultivars, as a result of different inoculum concentrations densities.

As for the weight of 100 fruits, the highest means obtained in cultivar Verdão were at concentrations densities, 0, 1,000, and 4,000 eggs/plant. In both cultivars Verdão and HTV Dom Luiz, the lowest fruit weight averages were obtained at concentrations densities of 8,000 and 16,000 eggs/plant, due to the death of the plants caused by the intense attack of the pathogen (Figure 2).

In several crops, the production reduction is reported due to the presence of *M. incognita*, as in the case of soybeans, where losses of 20% to 30% of production are were estimated [26]. However, in the present work, it was observed that for both the number of fruits and the weight of the 100 fruits, the presence of the pathogen up to the concentration density of 4,000 eggs/plant did not influence the production and productivity when compared to the control, concentration density of 0. Possibly, after transplantation to the 2 L pots, plants were able to reestablish the root system and shoot development, not interfering with the flowering and fruit filling. By means of these results, depending on the intensity of the pathogen in a given area of cultivation, it is possible to produce coriander fruits in soils contaminated by *M. incognita* race 1.

As for pollen viability, acetic Carmine and Alexander dyes reacted to the pollen grains, and consequently staining them. As for the tetrazolium solutions, regardless of concentrations (1%; 0.75%; 0.50% and 0.25%), they did not stain the pollen at any intensity of red (Figure 3).

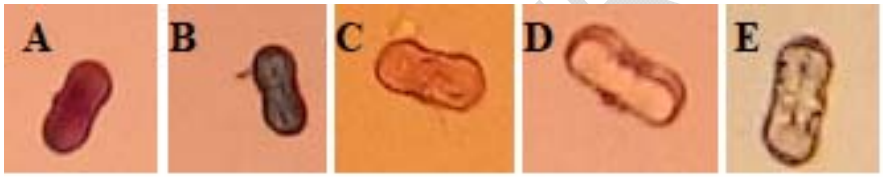


Figure 3. Coriander pollen grains stained with different dyes. Alexander solution, A) viable pollen grain, B) non-viable pollen grain. Acetic Carmine, C) viable pollen grain, D) non-viable pollen grain. Tetrazolium solution, E) non-viable pollen grain.

The results obtained for the tetrazolium solutions did not corroborate other studies with the same concentrations used in the present study for eggplant [18] and even in concentrations well below as 0.075% in wild passion fruit [27], where tetrazolium was efficient to stain the viable pollens. It is possible that there is some impediment to the penetration of tetrazolium in the structure of the coriander pollen grain, since according to [28], the tetrazolium test is reliable in distinguishing viable and non-viable pollen due to the reaction with the enzyme dehydrogenase of the malic acid that reduces the tetrazolium salt in living tissues where there are ions of H^+ forming a red compound, being related to the cellular respiration of the pollen.

As fruit yield was verified in the present study, and the plants were managed in a greenhouse where there was no entry of pollinators, it is possible to state that the fruits were obtained from pollination (possibly self-pollination) with the pollens from evaluated plants. Thus, the tetrazolium solutions used were not efficient in distinguishing viable and non-viable pollens in coriander.

The analysis of variance showed triple interaction (cultivars X inoculum concentrations densities X dyes) significant at 1% probability. The experimental coefficient of variation was

UNDER PEER REVIEW

5.28% (Table 4). The value obtained for the CV% approached that obtained by Santos et al. (2016), which was 3.82%, being considered a low CV% [29].

Table 4. Summary of variance analysis of the percentage of stained pollen grains from two coriander cultivars inoculated with four inoculum concentrations densities of *Meloidogyne incognita* race 1.

SV	DF	MS
		% of stained pollen grains
Block	3	26.96
Genotypes	1	237.62**
Concentrations	3	95.52*
Dyes	1	22.56ns
Genotypes*Concentrations	3	171.36**
Genotypes*Dyes	1	19.51ns
Concentrations*Dyes	3	97.53*
Genotypes*Concentrations*Dyes	3	181.42**
Error	45	25.10
CV (%)		5.28
Mean		94.82

* Significant at 5% probability
** Significant at 1% probability
ns Not significant.

There were significant differences between the cultivars when developed within concentrations densities of inoculums and dyes, only for the acetic Carmine dye at the concentration density of 1,000 eggs plant, where the HTV Dom Luiz cultivar had a higher percentage of stained pollen grains and were, therefore, considered viable (Table 5).

Table 5. Scott-Knott's grouping test of the percentage of pollen grains stained with two dyes of two coriander cultivars inoculated with four inoculum concentrations densities of *Meloidogyne incognita* race 1 grown under greenhouse conditions.

Cultivars	Inoculum Concentrations Densities	Dyes	
		Acetic Carmine	Alexander
Verdão	0	96.50 a A β	93.67 a A α
	1,000	75.00 a A α	94.50 b A α
	2,000	97.67 a A β	95.50 a A α

UNDER PEER

		4,000	97.83 a A β	92.50 a A α
HTV	**Dom**	0	97.00 a A α	97.00 a A α
Luiz		1,000	99.17 a B α	96.33 a A α
		2,000	92.50 a A α	95.67 a A α
		4,000	98.16 a A α	98.17 a A α

Means followed by the same lowercase letter in the row, upper case for cultivars and Greek for inoculum cultivars in the column do not differ statistically by the Scott-Knott test at 5% probability.

In the decomposition of concentrations densities within the cultivars and dyes, only the concentration density of 1,000 eggs/plant presented a lower percentage of pollen grains stained in relation to the other concentrations densities in the cultivar Verdão with acetic Carmine dye.

As for the dyes, there was variation within the cultivar Verdão at the concentration density of 1,000 eggs/plant, where acetic Carmine showed a smaller number of grains stained in relation to Alexander. In the study with cane-do-brejo, the lowest values of pollen stained for acetic Carmine and Acid Fuchsin were also observed [7].

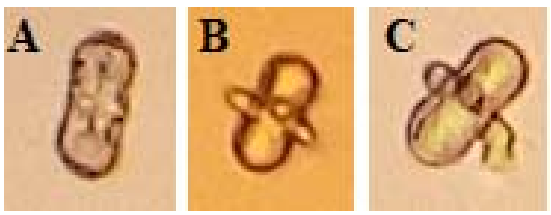
Due to the obtained results, both tested dyes can be used, independently of the concentration density of the inoculum used, because the lowest percentage of viable pollen was 75% obtained for the cultivar Verdão at the concentration density of 1,000 eggs/plant. [30], when working with passion fruit, an allogamous species, consider pollen viability high when values above 70% are obtained, where there is no compromise of breeding work.

In the evaluation of the pollen *in vitro* viability, none of the used media allowed pollen germination. The media were tested in the order in which they are found in Table 1. Initially, the means proposed by [31] and [18] were tested. As none of the means presented caused development of the pollinic tube, mean adaptation was tested in order to verify one that promoted pollen germination, for several factors influenced the germination of pollen *in vitro* from of which the temperature and period of incubation to the micro and macronutrients in the culture medium, according to [32].

In At the first alteration (C medium), the beginning of the pollen tube elongation was observed after 24 hours of incubation at 25°C. In order to verify if a longer incubation period was necessary, the evaluation was extended for a further 48 hours, observing every 24 hours, totaling 72 hours of observation. However, there was no progression of the pollen elongation, and the tube length obtained was insufficient to consider the pollen as germinated, considering the criterion proposed by [33] in which the germinated pollen must have a pollen tube length equal to or greater than the diameter of the pollen itself.

In order to identify an efficient medium in the germination of coriander pollen grains *in vitro*, adaptations were made in the C medium, varying in the concentrations of salts, sucrose, boric acid and phytagel, since according to [34, the types and concentrations of sugar and boron are important in the composition of the culture medium. Although G, I and J media showed the beginning of development of the pollen tube, there was no further growth (Figure 4).

347



UNDER PEER REVIEW

Figure 4. Evaluation of the development of the pollen tube cultivated *in vitro*. A) Absence of tube emission, result obtained for the most of the media tested; B) beginning of development of the pollen tube, result obtained in G, I and J media; C) elongation of the pollen tube, observed in C medium.

Based on the results obtained, there is a need for new studies that seek to identify the factor that is making it impossible to develop the *in vitro* pollen tube of coriander pollen grains. C medium, presented in this study, can be used as base medium, altering both the sugar and boron as well as the other salt concentrations, and the pH values of the medium. In addition to the concentrations of sugar and boron, pH is a factor that influenced the germination of pollen, a fact verified by [31] evaluating media with 3.5 - 6.5 pH in citrus, who verified a linear growth of the number of pollen grains germinated as the pH increased up to the level of 6.5 for the cultivars Pêra and Natal. However, in at the cultivar Valencia, the best results were obtained at pH around 5.

5. CONCLUSION

There is a possibility of inoculation in by the egg sowing of 1,000-4,000 eggs of *M. incognita* race 1 /cells or plant of to evaluate the reaction of coriander genotypes, allowing the quantification of galls and transplantation of the selected plants into pots, directing them to the recombination in open field, since the evaluation based on the number of galls is as a non-destructive method and the presence of the pathogen did not compromise the pollen viability and coriander fruit yield in the surviving plants evaluated.

Seed inoculation and evaluation at 30 days post inoculation provides a 50% reduction of the time required in the selection procedures in which inoculation is performed at 15 days after sowing and evaluation at 45 days post inoculation. In addition, the evaluation through the number of galls - in the early cycles - allowed the recombination in the same cycle of the selection, making possible the realization of three selective cycles in a year, thus, reducing cost and time to obtain superior genotypes resistant to the nematode.

Among the dyes used to verify pollen viability, acetic Carmine and Alexander solution were efficient in differentiating viable pollens from nonviable ones, and could be used to verify pollen viability in coriander. None of the tetrazolium concentrations stained the coriander pollen.

The culture media used for *in vitro* germination of the pollen grains did not allow the development of the pollen tube, and new studies must be continued in order to adjust a suitable medium for the coriander culture. C medium becomes became an option for continuation.

REFERENCES

1. Abad P, Gouzy J, Aury J-M, Castagnone-Sereno P, Danchin EGJ, Deleury E, Perfus-Barbeoch L, et al. Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita.* Nature Biotechnology 2008;26: 909-915.

UNDER PEER REVIEW

2. Ferraz LCCB, Brown DJF. Plant Nematology: fundamentals and importance. Manaus: Norma Editora. 251 p. 2016.

3. Engler JA, Siqueira KMS, Nascimento DC, Costa TG and Engler GA. Cellular outlook of galls induced by root-knot nematodes in the model host *Arabidopsis thaliana*. Nematoda 3. 2016.

4. Marschner H. Mineral nutrition of higher plants. London: Academic. 889p. 1995.

5. Pio LAS, Ramos SD, Pasqual M, Junqueira KP, Santos FC and Rufini JCM. Sweet orange pollen viability under different storage conditions. Science and Agrotechnology 2007;31: 147-153.

6. Oliveira MSP, Maves MM and Kalume MAA. *In vivo* and *In vitro* pollen viability in açaí plant genotypes. Acta Botanica Brasilica 2001;15: 27-33.

7. Santos BNV, Macedo WA, Mello VS, Damasio JF, Santos LCB, Leite DM and Karsburg IV. Do-brejo cane pollen grain viability estimate based based on different coloring methods. Science & Technology 8: n.1. 2016. Special issue

8. Santos AMM, Costa KDS, Silva J, Pereira JWL, Menezes D and Carvalho Filho JLS. Methodology for the genetic improvement of coriander aiming for resistance to the root knot nematode. Journal of Experimental Agriculture International 2018;20: 1-10.

9. Biondi CM, Prado MDC, Medeiros JE, Pedrosa EMR and Moura RM. Coriander tolerance to *Meloidogyne incognita* nematode parasitism Race 1. Brazilian Nematology 2001;25: 239-241.

10. Diniz GMM. Coriander resistence to (*Coriandrum sativum* L.) *Meloidogyne incognita* (Race 1 and 3) and *Meloidogyne javanica*. Dissertation presented to the “Genetic Breeding of Plants” Graduate program in Agronomy – UFRPE. 56 p. 2012.

11. Diniz GMM, Carvalho Filho JLS, Gomes LAA, Oliveira CL, Chagas WFT and Santos LS. Reaction of coriander cultivars to gall nematodes. Agricultural Science 2018;16: 61-68.

12. INMET – Instituto Nacional de Meteorologia (2018) Available at: http://www.inmet.gov.br/projetos/rede/pesquisa/gera_serie_txt_mensal.php?&mRelEstacao=82900&btnProcesso=serie&mRelDtInicio=01/07/2017&mRelDtFim=31/10/2017&mAtributos=,,,,,,,,,,,1,,1,,. Accessed in April, 2018.

13. Hussey RS and Barker KR. A comparasion of methods of collecting inocula of *Meloidogyne* spp. including a new technique. Plant Disease Reporter 1973;57: 1025 – 1028.

14. Bonetti JIS and Ferraz S. Modifications of the Hussey & Barker method for egg extraction of *Meloidogyne exigua* in coffee roots. Brazilian Phytopathology 1981;6: 553.

15. Furlani PR, Silveira LCP, Bolonhezi D and Faquin V. Hydroponic cultivation of plants. Campinas, Instituto Agronômico de Campinas IAC. 52 p. 1999. (Boletim Técnico, 180).

16. Kearns CA and Inouye DW. Techniques for pollination biologist. University of Colorado, Niwot. 583p. 1993.

17. Alexander MP. A versatile stain for pollen, fungi, yeast and bacteria. Stain Technology 1980;55: 13-18.

UNDER PEER REVIEW

18. Tatis HA, Ayala CC and Orozco AJ. Efficiency of two methods to evaluate the viability of eggplant pollen (*Solanum melongena* L. cv. Lila criaola). U.D.C.A Journal News & Scientific Disclosure 2013;16: 351 – 358.

19. Munhoz M and Luz CFP. Meissner Filho, P. E. Barth, O. M. Reinert, F. *Carica papaya* L. pollen viability: a methodological comparison. Brazilian Journal of Botany 2008;31: 209-214.

20. Astudillo MJ. Evaluation of pollen conservation methods subject to different tomato storage times (*Lycopersicon esculentum* Mill). Ed. Pontificia Universidad Católica de Valparaiso. 54p. 2006.

21. Hoekstra FA and Bruinsma J. Respiration and vitality of binucleate and trinucleate pollen. Plant Physiology 1975;34: 221-225.

22. Iborra JL, Guardiola J, Montaner S, Canovas M and Manjon A. 2, 3, 5 Triphenyltetrazolium chloride as a viable assay for immobilized plan cells. Biotechn. Techn. 1992;6: 319-322.

23. França Neto LV, Nascimento W, Carmona RE and Freitas R. Viability of eggplant pollen. Crop Breeding and Applied Biotechnology 2009;9: 320-327.

24. Ferreira DF SISVAR: a program for analysis and teaching of statistics. Scientific Journal Symposium 2011;6: 36-41.

25. Correia ECSS. Reproductive potential and damage caused by *Meloidogyne incognita, M. javanica* and *M. enterolobii* in beet. Thesis (Doctorate) – Paulista State University, Agronomy Sciences College, Botucatu.69 p. 2017.

26. Asmus GL. Damage caused to the soybean crop by nematodes of the genus *Meloidogyne*. In: SILVA, J. F. V. (Org.). Host-parasite relationships in soybean meloidogynes. Londrina: Soy Embrapa/Brazilian Nematology Society 2001;39-62.

27. Santos LCB, Fernandes L, Damasio JF, Mello VS, Leite DM, Karsburg IV and Praça-Fonte MM. Use of tetrazolium to test pollen viability in wild passion fruit. Science & Technology 8: n.1. 2016 a. Special Issue 2.

28. Santos BNV, Macedo WA, Mello VS, Damasio JF, Santos LCB, Leite DM and Karsburg IV. De-macaco cane pollinic viability estimate based on the use of 2,3,5 triphenyltetrazolium chloride. Science & Technology 7: 2016 b. Special Issue.

29. Pimentel-Gomes F. Experimental statistics course. Nobel, Piracicaba, 468p. 1990.

30. Ruggiero C, São José AR, Volpe CA, Oliveira JC, Durigan JF, Baumgartner JG, Silva JR, Nakamura K, Ferreira ME, Kavati R and Pereira VP. Passion fruit for export: technical aspects of production. Brasília: EMBRAPA-SPI. 64 p. 1996.

31. Salles LA, Ramos JD, Pasqual M, Junqueira KP and Silva AB. Sucrose and pH in the *in vitro* germination of citrus pollen grains. Science Agrotechnology 2006;30: 170-174.

UNDER PEER REVIEW

506 32. Taylor LP and Hepler PK. Pollen germination and tube growth. Annual Review of Plant
507 Physiology and Plant Molecular Biology 1997;48: 461-91.
508
509 33. Pasqual M, Petri JL and Mattos CS. Pollination of apple plant III: cultivars BR-1 and
510 Mollies Delicious. Brazilian Agricultural Research 1982;17: 1477-1481.
511
512 34. Miranda PA and Clement CR. Germination and storange of pejibaye (*Bactris gasipaes)*
513 palmae pollen. Revista de Biologia Tropical 1990;38: 29-33.
514
515

UNDER PEER REVIEW