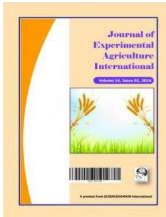

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Solanum melongena L. Ecophysiology under the Influence of *Meloidogyne javanica*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FRAF, JSN and JESR designed the study, performed the statistical analysis and wrote part of the manuscript. Authors TIS, FJS and RGSN collaborated in the implementation and evaluation of the data. Authors MBA, GSP and RLAB collaborated in the development of the study and made the corrections of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The purpose herein was to quantify the ecophysiological exchanges of eggplants cv. Embu (*Solanum melongena* L.) conducted with and without *Meloidogyne javanica*.
Study Design: The experimental design has completely randomized with two groups of plants (with and without soil infestation) with five replications and two plants per pot (treatment).
Place and Duration of the Study: The experiment has carried out at the Agrarian Sciences Center of the Federal University of Paraíba, Areia, PB, between October to December, 2017.
Methodology: The eggplant cultivar employed was 'Embu'. Its seedlings were transplanted to 5

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dm^3 pot, filled with a substrate formulated by the mixture of vegetal soil, sand, and cattle manure in the proportion of 3:1:1.Their growth characteristics were valuated after 60 days of transplant (DAT), when the following variables were measured: plant height; stem diameter; leaf number; flower number; and aerial part, root, and total dry masses; and Dickson quality index. Gas exchange evaluations were performed between 7:00 a.m. to 5:00 p.m. The level of chlorophyll under fluorescence emission was measured several times.

Results: There was no significant difference for the variables, growth, fluorescence and chlorophyll between plants with and without *M. javanica* inoculation, which proved that they were resistant to the population level to which they were encountered. Plants inoculated with *M. javanica*, there was a greater increase of the CO_2 assimilation rate and in the carboxylation efficiency.

Conclusion: Therefore, we can say that these plants have mechanisms to control their photosynthetic activities, which make them resistant to *M. javanica* stress, avoiding growth damages.

Keywords: Root-knot nematodes; gas exchange; physiological behavior.

1. INTRODUCTION

Eggplant (*Solanum melongena* L.) is a species that belongs to the Solanaceae botanical family. It originated from countries of tropical eastern regions, such as China and India, presenting an elevated economic importance due to its nutraceutical properties [1]. The increasing demand of the consumer for this vegetable is noteworthy. One of the possible explanations for such increase is that, besides the fact that consumers are searching for healthier products, eggplants have proven nutritional and medicinal properties [2]. Additionally, eggplants contain lowering cholesterol compounds and are widely used as an alternative treatment for plasma cholesterol [3,4].

Generally, vegetables are usually affected by diseases caused by phytopathogenic microorganisms [5]. Although considered a tough species, eggplants are sensible to pathogens attacks, which, without proper care or control, may cause damages and change the final product quality [6].

The main features of nematodes of the genus *Meloidogyne* are the formation of knots in the cultures' root system. Especially in the eggplant culture, they have already become one of the most important pathogens due to their nature of difficult control [7]. When conducted under high population densities, the cultures' photosynthetic rate, growth, and production tend to decrease [8].

Before these facts, there is little information about the olerocarpa plant's physiological activity, especially eggplants, under the influence of soil pathogens, which justifies this research. There is also little information on the behavior of plants in their different phenological stages when exposed to different root-knot nematode population densities.

This information may be useful for the culture genetic improvement regarding these pathogens attack due to their nature of difficult control. Therefore, the purpose herein was to quantify the ecophysiological changes of eggplants (*Solanum melongena* L.) conducted with and without *Meloidogyne javanica*.

2. MATERIALS AND METHODS

The research has carried out in a greenhouse belonging to the Department of Plant Science and Environmental Sciences of the Federal University of Paraíba, UFPB, located in the city of Areia, Paraíba, Brazil.

The experimental design was completely randomized with two groups of plants (with and without nematode soil infestation) with five replications and two plants per pot. In order to obtain the inoculum, root-knot nematode, *Meloidogyne javanica* population was maintained and multiplied on tomato plants (*Solanum lycopersicum* L. var. Santa Clara), were grown in pots with a capacity of 2 dm^3 of ravine soil and sand (2:1), and maintained in a greenhouse during the period of 70 days.

The eggs extraction was performed according to the methodology suggested by Hussey and Barker [9] and adapted by Boneti and Ferraz [9]. The aerial part of the tomato plants was separated from its root system, and the roots were washed, cut, and ground in a blender in a 0.5% sodium hypochlorite (NaClO) solution at low rotation for 20 seconds. The solution was refined through 200- and 500-mesh (holes of 74 and 26 mm, respectively) sieves. The contents of the 500-mesh sieve were washed with water to remove all sodium hypochlorite, collected in a beaker, and quantified with the assistance of an optical microscope. Then, the soil has been inoculated with 6,000 *Meloidogyne javanica* eggs per pot (experimental unit).

Eggplant cultivar employed was Embu (Feltrin®). The seedlings were produced in polyethylene trays with a commercial substrate (Basaplant®). Three seeds were sown per cell, and the excess seedlings were removed after their emergence. The transplant was performed when the plants reached 10-15 cm height, and the seedlings were transplanted to 5 dm^3 pots, filled with a substrate formulated by the mixture of vegetal soil, sand, and cattle manure in the proportion of 3:1:1, pre-sterilized in an autoclave under the temperature of 120ºC and 1 atm of vapor pressure for 2 hours. The irrigations were performed whenever necessary, maintaining the soil moisture close to the field capacity.

The growth characteristics were evaluated after 60 days of transplant (DAT), when the following variables were measured: plant height, using a ruler graduated in centimeters, measuring from plant base to its apical bud; stem diameter, performed with the assistance of a digital caliper, whose results are expressed in mm. Leaf number was established by counting the leaves, considering photosynthetically active leaves; flower bud number was established by counting the number of buds found on the plant. The aerial part, root, and total dry masses were measured at the end of the experiment through the separation of the aerial and radicular parts at the level of the plant's base, which was stored in Kraft paper bags and dried in a forced air circulation muffle until they reach a constant weight. Then, they were weighed in a 0.001g accuracy analytical scale. The total dry mass was determined through the sum of the aerial part and root dry masses. The results were expressed in g $plant^{-1}$; the Dickson quality index was established by the plant height (H), stem diameter (SD), aerial part and root dry masses, according to Dickson et al. [10].

$$\mathrm{DQI} = \frac{\mathrm{TDM}}{\mathrm{H\ (cm)/SD\ (mm)} + \mathrm{APDM\ (g)\ /\ RDM\ (g)}}$$

Where: TDM = total dry mass; H = height; SD = stem diameter; APDM = aerial part dry mass; RDM = root dry mass.

Physiological evaluations were performed on the third leaf from the apex, from 7:00 a.m. to 5:00 p.m., with 2-hour intervals, totalizing six evaluations. The infrared gas analyzer (IRGA, LI-COR® 6400-XT, Lincon, USA) was employed to evaluate the CO_2 assimilation rate (A) (µmol CO_2 m^{-2} s^{-1}), transpiration (mmol H_2O m^{-2} s^{-1}), internal CO_2 concentration (iC) (µmol CO_2 mol^{-1}), stomatal conductance (gs) (mmol H_2O m^{-2} s^{-1}), and leaf temperature (ºC). After the data collection, the water use efficiency (WUE - A/E), intrinsic water use efficiency (iWUE - A/gs), and carboxylation instantaneous efficiency (CiE – A/iC) were quantified.

The chlorophyll *a* fluorescence emission measurements were performed according to the method described by Melo et al., using the OS-30p+ (Optisci) fluorometer model, establishing the initial (F_0), maximum (F_m), and variable(F_v = F_m - F_0) fluorescences, and the potential quantum yield of the photosystem II (PSII = F_v/F_m). The establishment of the chlorophylls *a*, *b*, and total chlorophyll rates was performed using a non-destructive method with a portable chlorophyll meter (ClorofiLOG®, model CFL 1030). The rates were given in Falker chlorophyll index (FCI).

The data were submitted to analysis of variance, and the means were compared by Tukey test, at a 5% level of significance. In the significance cases, polynomial regression analyses were performed using the SAS University statistical program [11].

3. RESULTS AND DISCUSSION

Generally, the *Meloidogyne javanica* density inoculated in the eggplants (*Solanum melongena* L.) in the present experimental conditions did not cause significant changes in growth variables (Table 1). The results suggest that the eggplant is a very tough species, exhibiting an elevated resistance to this pathogen.

Generally, the formation of root-knots in eggplant roots occurs in a smaller dimension in comparison with other plant species, such as tomatoes (*Solanum lycopersicum*), chilli peppers (*Capsicum* spp.), and peppers (*Capsicum annum*) [7]. This, which might suggest that this species resists certain nematode population levels because when they infect roots, the symptoms affect the plant's growth, which was not observed in this study.

Table 1. Chemical characteristics of the substrate components used in the experiment

pH	P	K^+	Na^+	$H^+ + Al^{+3}$	Al^{+3}	Ca^{+2}	Mg^{+2}	SB	CEC	OM
	--- mg kg^{-3} ---		---------------------- cmol$_c$ dm^{-3} ---------------------------							g kg^{-1}
6.1	79.68	252.02	0.11	2.62	0.0	3.32	1.98	6.06	8.68	23.58

SB = Sum of bases; CEC = Cation exchange capacity; OM = Organic matter

Table 2. Mean values obtained for the variables plant height (PH), stem diameter (SD), leaf number (LN), flower buds (FB), root dry mass (RDM), aerial part dry mass (APDM), total dry mass (TDM), and the Dickson quality index (DQI) in eggplants with nematodes(WN) and without nematodes (WON)

Variation sources	Variables analyzed							
	PH	SD	LN	FB	RDM	APDM	TDM	DQI
WN	52.1 a	10.49 a	25.6 a	10.2 a	65.32 a	26.11 a	91.43 a	92.90 a
WON	48.1 a	10.45 a	22.2 a	8.2 a	42.84 a	25.35 a	68.19 a	69.36 a
CV (%)	10.53	9.90	18.47	32.96	30.21	12.67	21.67	21.71

Means followed by the same letters in the column do not differ from each other by Tukey test up to 5% of probability

Nóia et al. [12], evaluating onion (*Allium cepa*) and eggplant genotypes, reported that they were proved resistance to *M. javanica*, that corroborate herewith. As for Ribeiro et al. [13], they got different values, reporting that the 'Embu' eggplant is susceptible to this nematode, with a great number of root-knots and eggs in its roots. This divergence regarding the present work may be related to that this cultivar resisted to the *M. javanica* breeding used herein.

As for the variables initial, variable and maximum fluorescences, quantum efficiency of PSII, variable and initial fluorescence ratio, chlorophylls *a*, *b*, and total were not affected by the soil nematode infection (Table 3).

When the F_v/F_m values are below 0.75 quantum^{-1} electrons, the plants are under stress, and when they were between 0.75 and 0.85 quantum^{-1} electrons, the plants are properly performing their photosynthetic activities [14]. A decrease in the F_v/F_m values may be related to a deviation of part of photons to the fluorescence pathway rather than to the photochemical pathway. If this happens, the plants can reduce their photosynthetic processes. In this work, we noticed that, based on these variables, the plants are not under stress, which can be confirmed by the fact that the chlorophyll index did not change either.

This behavior might happen due to defence mechanisms that are triggered in the plants, mitigating or preventing that the nematode attack cause physiological and biochemical disturbances in the plants. The plants' resistance to nematodes of the *Meloidogyne* sp. is developed through mechanisms that comprise periods established during and after the nematode penetration into the roots [15].

Plants resistance to nematodes may be established as a series of characteristics developed by the plant in detriment to the parasitism, which may be manifested as mechanical barriers, or physiological or chemical changes, which prevent the nematodes from entering the plant tissues [16]. The same authors also stated that the plants can promote changes in their life and reproduction cycles in response to nematode attacks.

Table 3. Mean values obtained for the variables initial (F_0), variable (F_v), and maximum (F_m) fluorescences, quantum efficiency of PSII (F_v/F_m), variable and initial fluorescence ratio (F_v/F_0), chlorophyll *a* (C*a*), chlorophyll *b* (C*b*) and total chlorophyll (tC) in eggplants with nematodes (WN) and without nematodes (WON)

Treatments	Variables analyzed							
	F_0	F_v	F_m	F_v/F_m	F_v/F_0	C*a*	C*b*	tC
WN	67.00 a	235.6 a	302.6 a	0.77 a	3.51 a	38.71 a	19.38 a	58.09 a
WON	72.20 a	234.0 a	306.2 a	0.76 a	3.34 a	38.16 a	20.78 a	59.02 a
CV (%)	13.71	6.02	2.74	4.01	15.52	3.60	14.96	6.94

Means followed by the same letter in the column do not differ by Tukey test up to 5% of probability

The resistance induction in plants to nematode attacks consists of a series of signals that change the parasite's activity. These changes are characterized by the interruption of parasitic cycle extension, which may directly or indirectly affect the hatching stimulus, the mobility of young parasites, their ability to penetrate into the host plant roots, and the maintenance of the nematodes' feeding sites [17]. This behavioral characteristic may have facilitated the plants resistance to this pathogens attack.

Based on the results, we may notice that the values of CO_2 net assimilation (A), stomatal conductance (gs), CO_2 internal concentration (iC), and transpiration (E) presented a quadratic response to the effect of different reading times (Fig. 1). As for the CO_2 net assimilation, we may notice that the greatest values were occurred in the first reading times, both with and without nematodes, reaching maximum values at 9:00 a.m., with 20.89 and 19.54 µmol CO_2 m^{-2} s^{-1}, respectively, gradually decreasing over the day (Fig. 1A).

This behavior can be explained by the fact that the temperature increase imposes a limit to the CO_2 assimilation rate due to the CO_2 solubility reduction and to the carboxylation reactions and RuBisCO oxidation ratio in C3 plants, which is the eggplant's case [18]. We can also notice that plants grown with nematodes presented a greater photosynthetic rate in comparison with the others, which justifies the statement that the eggplant is considered a rather tough plant, being more resistant to this type of stress than other species of the same family, such as tomato and pepper [19]. The fact that the plants increased their photosynthetic processes with nematodes can be related to the stress acclimatization phenomenon. It gives the plants a way to adjust their metabolic processes and deal with the stressful situations that are imposed on them. Similar results were reported by Mioranza [20], where the main symptom caused by nematodes of the genus *Meloidogyne* in tomatoes was the increase of the photosynthetic rate.

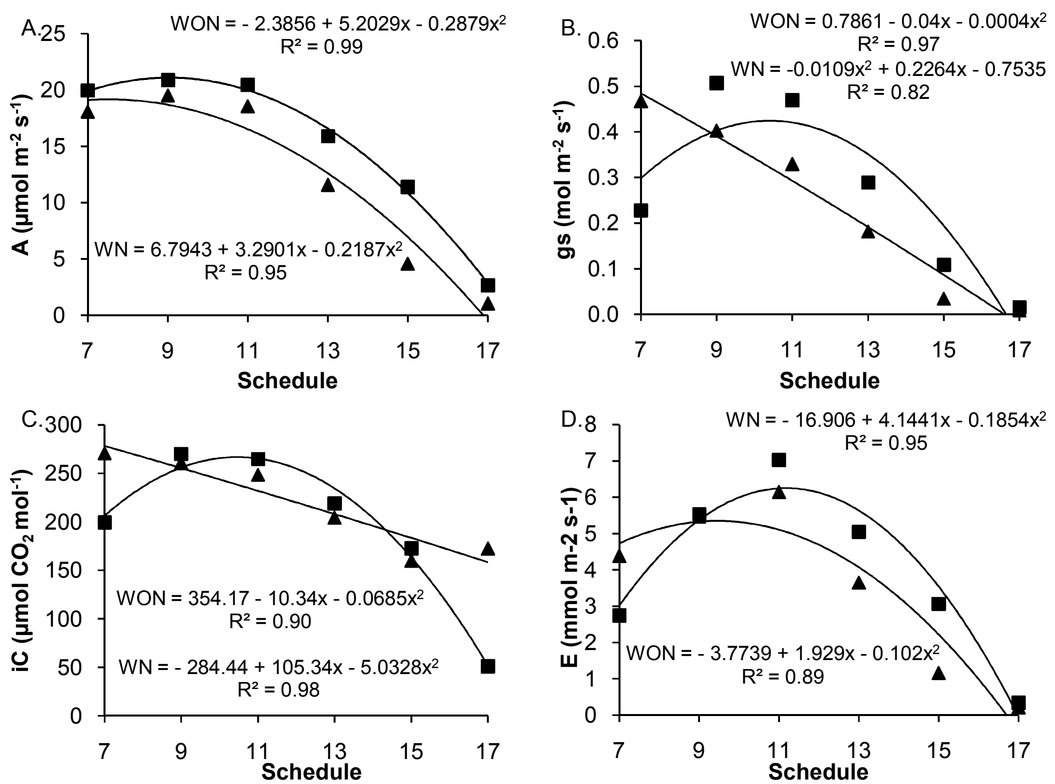


Fig. 1. Daily variation of CO_2 net assimilation rate (A), stomatal conductance (gs) (B), CO_2 internal concentration (iC) (C),and transpiration (E) (D) in eggplants (*Solanum melongena* L.) with (WN) and without (WON) nematodes

(■) with nematodes (▲) without nematodes

For the stomatal conductance, maximum values were registered at 9:00 a.m. for plants with nematodes, with 0.50 mol H_2O m^{-2} s^{-1}; without these pathogens, however, they presented the greatest increase at 7:00 a.m., with 0.46 mol H_2O m^{-2} s^{-1}, which decrease over time (Fig. 1B). This decrease might have happened due to the increase of temperature, which causes stomatal closures and, consequently, the decrease of the transpiration rate, thus avoiding the loss of water.

According to Magalhães Filho et al. [21], plants perform stomatal closures as the first line of defense when they are encountered to the same type of stress, which happens due to both dehydration of the guards cells and due to hormonal response. Endres [22], while working with *Annona squamosa*, got similar results, in which the stomatal conductance was greater in the first reading times and began to decrease after 8:00 a.m., which, according to the same author, might have happened due to a vapor pressure deficit (VPD) increase.

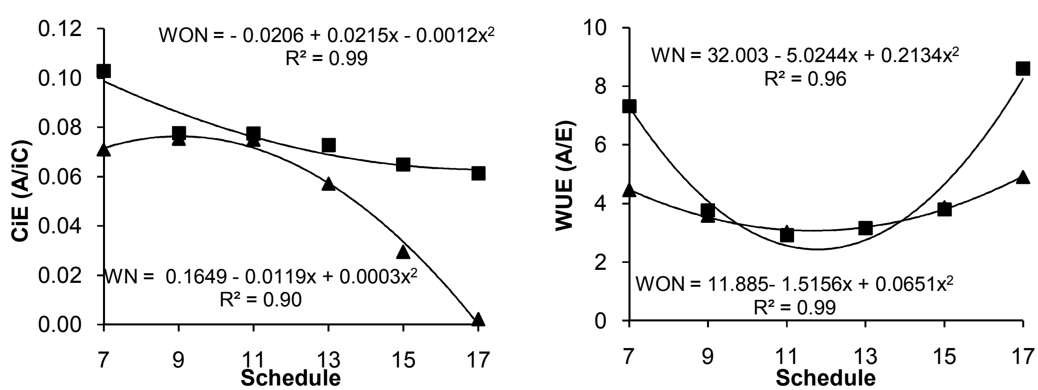
The CO_2 internal concentration follows the same stomatal conductance behavior, presenting a significant difference between the treatments, with maximum values of 270.32 and 270.95 µmol CO_2 mol^{-1} for plants grown with and without nematodes, respectively (Fig. 1C). This behavior, according to Silveira et al. [23], happens because the stomatal opening is related to the diffusion of CO_2. Machado et al. [24] also reported this change in orange trees, in which the CO_2 input in the leaf mesophyll decreased proportionally to the stomatal conductance.

Transpiration values also presented a behavior that was similar to the stomatal conductance, with maximum values of 7.03 and 6.14 mmol H_2O m^{-2} s^{-1} at 11:00 a.m. in plants with and without nematodes, respectively, which decreased over time (Fig. 1D). Machado et al. [24] stated that stomatal conductance is proportional to transpiration, supporting the hypothesis that bigger the stomatal limitation is, the lower the transpiration rate will be.

These results are similar to those found by Messchmidt [25], who stated that this stress can increase the production of abscisic acid (ABA) in the culture root system, and that this signal can be transmitted to the aerial part, which will cause the stomatal closure, promoting a decrease in the photosynthesis, transpiration, and stomatal conductance rates.

The values for carboxylation instantaneous efficiency (CiE), water use efficiency (WUE), intrinsic water use efficiency (iWUE), and leaf temperature (LT) were also adjusted to the quadratic effect (Fig. 2). As for carboxylation efficiency, the results were similar to CO_2 assimilation and intracellular CO_2 concentration, which presented greater increases at 7:00 a.m. for plants grown with nematodes, with 0.10 µmol m^{-2} s^{-1}, and at 9:00 a.m. for plants grown without nematodes, with 0.07 µmol m^{-2} s^{-1}, respectively (Fig. 2A). Ferraz et al. [26] reported that this behavior might have occurred due to the increases in the assimilation and intracellular CO_2 rates. This CO_2 fixation efficiency plays an important role in the vegetables' growth and aptitude [27].

Water use efficiencies behaved similarly over the day, with maximum increases at the end of the afternoon (Fig. 2B and 2C, respectively).It may be a reflection of the stomatal closure registered during the reading. Machado Filho et al. [28], working with papaya under field conditions, also



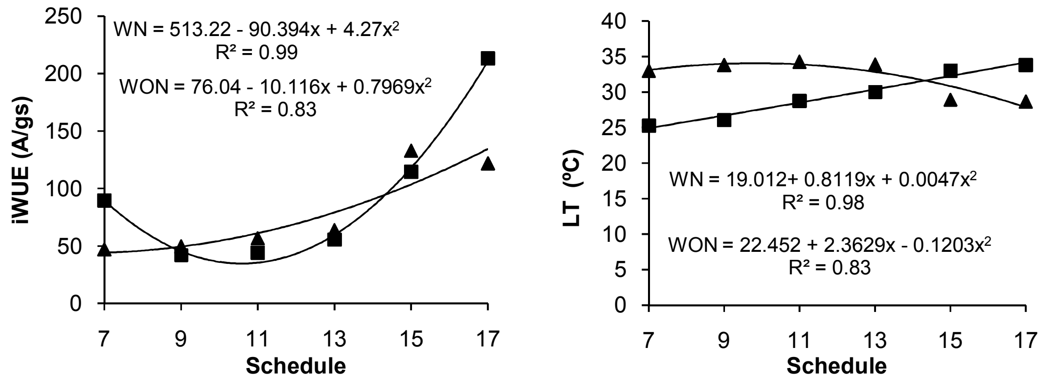


Fig. 2. Daily variation of carboxylation instantaneous efficiency (CiE) (A), water use efficiency (WUE) (B), water use intrinsic efficiency (WUiE) (C), and leaf temperature (LT) (D) in eggplants (*Solanum melongena* L.) with (WN) and without (WON) nematodes

(■) with nematodes (▲) without nematodes

reported values that are similar to the one presented in this study for water use intrinsic efficiency.

Foliar temperature presented a different behavior between plants grown with and without nematodes: the plants grown without nematodes usually had higher leaf temperatures in comparison with the others (Fig. 2D). We should be very careful while measuring it because this factor can represent a limiting factor for the photosynthetic process, that, extremely high leaf temperatures limit the RuBisCO activity [29]. Its oxygenase activity is increased at higher temperatures at the carboxylase activity expense, thus, increasing the photorespiration process and CO_2 loss.

4. CONCLUSION

- The presence of *Meloidogyne javanica* did not affect the eggplant (*Solanum melongena* L.) growth and flowering characteristics, as well their quantum efficiency of PSII, chlorophylls *a*, *b*, and total. However, it promoted their CO_2 net assimilation and carboxylation efficiency increase.
- Therefore, we can state that these plants have mechanisms to control their photosynthetic activities, which make them resistant to *M. javanica* stress, avoiding growth damages.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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