NEMATOFAUNA AND ARBUSCULAR MYCORRHIZAL FUNGI IN ORGANIC AND CONVENTIONAL VINE (Vitis vinifera L.) PLANTATIONS IN THE SUBMEDIUM SÃO FRANCISCO VALLEY

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ABSTRACT

Objective: to evaluate nematofauna and AMF present in organic and conventional vine plantations.

Theoretical Framework: vines are widely cultivated around the world. Arbuscular mycorrhizal fungi (AMF) associate with plants, providing an increase in nutrient absorption. Free-living soil nematodes participate in the trophic web, being important for its maintenance. These two organisms can be used as indicators of soil quality.

Materials and methods: Collection was carried out in the areas: organic vine cultivation, conventional vine cultivation and Caatinga. The following were evaluated: trophic groups of nematodes, number of glomerospores, percentage of mycorrhizal colonization.

Results: There was a significant difference between the areas regarding the amount of bacteriovorous, fungivorous and carnivorous nematodes. AMF spore density differed between areas, with the highest values observed in conventional vine planting.

Conclusions: conventional cultivation of vines negatively affects free-living nematodes; on the other hand, phytonematodes are favored by vine cultivation, regardless of the type of cultivation. AMF are affected by the type of cultivation, with greater production of glomerospores in conventional cultivation.

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Implications of the research: this research finds that the biological conditions of the soil tend to be different between organically managed and conventionally managed crops, where the latter cause a reduction in the number of organisms, such as nematodes, on the other hand, organic management tends to improve edaphic conditions. Another issue is that not all soil organisms will be affected in the same way, so those that are more resilient tend to adapt to the new conditions imposed, as seen in the present work in relation to AMF.

Keywords: Grapevine Cultivation, Soil Nematodes, Arbuscular Mycorrhizae, Soil Quality.

RESUMEN

Objetivo: evaluar la nematofauna y los HMA presentes en plantaciones de vid orgánicas y convencionales.

Marco teórico: la vid se cultiva ampliamente en todo el mundo. Los hongos micorrízicos arbusculares (HMA) se asocian con las plantas, proporcionando un aumento en la absorción de nutrientes. Los nematodos del suelo lo libre participan en la red trófica, siendo importantes para su mantenimiento. Estos dos organismos pueden utilizarse como indicadores de la calidad del suelo.

Materiales y métodos: La colecta se realizó en las áreas: cultivo de vid orgánico, cultivo de vid convencional y Caatinga. Se evaluaron: grupos tróficos de nematodos, número de glomerosporas, porcentaje de colonización micorrízica.

Resultados: se encontró una diferencia significativa entre las áreas en relación a la cantidad de nematoides bacterívoros, fungívoros y carnívoros. La densidad de esporas de HMA también varió entre las áreas, siendo los máximos valores observados en el cultivo convencional de vid.”
Resultados: Hubo diferencia significativa entre las zonas en cuanto a la cantidad de nematodos bacterióvoros, fungivoros y carnívoros. La densidad de esporas de HMA difirió entre áreas, observándose los valores más altos en la plantación de vid convencional.

Conclusiones: el cultivo convencional de vid afecta negativamente a los nematodos de vida libre; Por otro lado, los fitonematodos se ven favorecidos por el cultivo de la vid, independientemente del tipo de cultivo. Los HMA se ven afectados por el tipo de cultivo, siendo mayor la producción de glomerosporas en el cultivo convencional.

Implicaciones de la investigación: esta investigación encuenatra que las condiciones biológicas del suelo tienden a ser diferentes entre cultivos manejados orgánicamente y manejados convencionalmente, donde estos últimos provocan una reducción en el número de organismos, como los nematodos, por otro lado, el manejo orgánico tiende a mejorar las condiciones edáficas. Otra cuestión es que no todos los organismos del suelo se verán afectados de la misma manera, por lo que aquellos que son más resilientes tienden a adaptarse a las nuevas condiciones impuestas, como se ve en el presente trabajo en relación con los HMA.

Palabras clave: Cultivo de la Vid, Nematodos del Suelo, Micorrizas Arbusculares, Calidad Del Suelo.

1 INTRODUCTION

The vine (Vitis vinifera L.) is a fruit plant belonging to the Vitaceae family, being widely cultivated throughout the world. This vegetable produces fruits used in food. Among the cultivars produced, a large part of these are table grapes, with and without seeds, and some cultivars are grapes used for the production of juices and wines. In the São Francisco Valley region, the vine stands out as the second most produced fruit tree in the region (Silva and Correa, 2004; Gazzola et al., 2020), from where it is exported to several countries in Europe and Asia, and only part is sold in Brazil. These can be produced conventionally, using chemical fertilizers and pesticides, or they can be produced organically, using organic fertilizers and natural pesticides.

Arbuscular mycorrhizal fungi (AMF) associate with most terrestrial plants, providing an increase in the area of absorption of nutrients and water from the soil for the plant, which in return provides photosynthates necessary for the maintenance and reproduction of AMF. These fungi increase plant growth, as observed in grapevines (Freitas, 2006; Ye et al., 2023), as well as helping to reduce biotic and abiotic stresses suffered by these vegetables (Campos, 2020; Coninx et al., 2017).

Nematodes are animals with a vermiform body belonging to the phylum Nematoda, found in the most diverse environments, some species cause diseases in animals and others in plants, causing serious damage to agriculture and public health, and others are considered free-
living, occurring in water and soil in water films. Free-living nematodes are classified into: Omnivores, Bacteriovores, Fungivores and Carnivores (Bongers and Bongers, 1998).

Nematodes and AMF play important roles in the soil. The former participate in the trophic chain, being involved in nutrient cycling (Bongers and Bongers, 1998); while AMF stand out in maintaining ecosystems (Van der Heijden et al., 1998). Due to the role they play in ecosystems, these two organisms can be used as indicators of soil quality, influencing and being influenced by various environmental factors.

Some studies show differences related to these organisms in different areas (Campos, 2009; Pen-Mouratov et al., 2004). Therefore, the hypothesis of this work is that the quantity and/or type of these organisms in the soil differ between areas of vines cultivated organically and conventionally cultivated, indicating the soil conditions of the areas evaluated.

2 OBJECTIVES

Thus, the objective of the present work was to evaluate the nematofauna, through identification and quantification of trophic groups, and the AMF, after evaluating mycorrhizal colonization and spore density, present in organic and conventional vine plantations.

3 THEORETICAL FOUNDATION

3.1 THE VINE

The vine plays an important commercial role, whether through its use as table grapes (in natura) or through its use in the production of wines and juices. Viticulture is a very ancient practice, excavations carried out in the ancient commercial city of Kannish in Turkey show that the practice of this cultivation dates back to the Bronze Age, around 3,500 years BC (Soares and Leão, 2009), from where it spread across several European countries. In Brazil, the vine arrived around 1532 brought by the Portuguese, but became important with the arrival of Italian immigrants in the 19th century (Filho, 2011).

The best development of the vine occurs in regions with a Mediterranean climate. But it adapts well to different climatic conditions. The plant prefers temperatures between 15 and 30 degrees, a range that influences the photosynthesis process, productivity and the length of days between flowering and harvest, a period that must have a lot of light. With the exception
of waterlogged areas, the vine does well in any type of soil. In addition to climate and soil requirements, grape cultivation requires high technical knowledge from the producer or qualified technical assistance. With regard to labor, which is abundant in the entire production process, it is necessary to be able to correctly carry out all tasks relating to this culture (Filho, 2011).

Brazil ranks 14th in global grape production, with China and Italy being the largest producers of this fruit worldwide. Most of Brazil's grape production comes from the South region and in the Northeast the largest production is found in the Vale do Submédio São Francisco region, which covers the cities of Petrolina in the state of Pernambuco and Juazeiro in the state of Bahia (Filho, 2011; Gazzola et al., 2020).

The Region is privileged because it produces grapes all year round and thus takes advantage of the best price conditions when other producing Regions are not producing. The Northeast has also increased the production of seedless grapes, which have excellent export value (Filho, 2011; Gazzola et al., 2020). The vine is grown both conventionally and organically.

In the Brazilian semi-arid region, the practice of grafting is the most advisable as it can provide better adaptation to soils with low fertility and vigor against soil diseases such as nematodes (EMBRAPA, 2015). The most used cultivars in this region are Itália or Pirovano 65, Red Globe, Brasil, Sweety Globe, Thompson Seedless or Sultanina, Benitaka, Sugraone, and Crimson Seedless (EMBRAPA, 2015). To cultivate the vine there are two annual seasons considered ideal, the first is between the months of July and August and the second is between October and December. The spacing between plants should be between 2x2 and 3x3 meters and it is advisable to avoid places with a high incidence of strong winds, the use of irrigation is more advisable to obtain good production.

3.2 CULTIVATION MODES

The conventional method consists of the use of chemical fertilization and chemical fertilizers, with the aim of producing better quality fruits that are more resistant to pests, and reducing damage caused by pathogens and/or disease-causing microorganisms; however, the excessive use of these chemical inputs can cause harm to the soil and to humans as well, and
can lead to the impoverishment of the soil microbiota (Souza et al., 2024; Ecycle, 2015; Seufert et al., 2012).

The organic cultivation method aims at sustainable use of the soil through the use of organic matter, which does not harm the environment or humans. This practice can also favor the soil microbiota, by providing better aeration and favoring the increase of microbial activity and the decomposition of organic materials (Zanatta et al., 2022; Ecycle, 2015; Seufert et al., 2012).

3.3 ARBUSCULAR MYCORRHIZAL FUNGI

Mycorrhizae are associations between plant roots and certain fungi in the soil, in these relationships the plant provides photosynthates to the fungus, which in return provides mineral nutrients from the soil, also providing better water absorption. Most angiosperms, gymnosperms and pteridophytes and numerous bryophytes form mycorrhizae (Saggin Júnior and Silva, 2006; Varma et al., 2017).

Mycorrhizae are divided into two groups: (a) ectomycorrhizae, which is characterized by the intercellular penetration of hyphae into the host cortex; (b) endomycorrhizae, characterized by the intercellular and intracellular penetration of their hyphae and the formation of specialized structures. Among the endomycorrhizae we find arbuscular, formed by fungi from the phylum Glomeromycota (Schüßler et al., 2001; Tedersoo et al., 2018), which are associated with most land plants. It is characterized by the formation of arbuscules, structures whose function is to exchange nutrients between the symbionts.

The phases of symbiosis can be divided into the pre-symbiotic phase, where there will be the first contact of the fungus with the roots of the plant through hyphae arising from the germination of the spore. After this, the symbiotic phase takes place where the formation of the appressorium and the penetration of hyphae inter and intracellularly and the formation of arbuscules occurs, with symbiosis actually occurring and finally the hyphae grow outside the roots forming the extraradicular mycelium, in which new cells will be formed. Spores that will continue the AMF life cycle (Paszkowski, 2006)

Symbiosis occurs through a series of chemical interactions between the host and the fungus, with the participation of several hormones (Harrison, 2012; Saggin Júnior and Silva, 2006). These fungi bring beneficial effects to plants, such as improving growth and fruit production (Campos, 2009; Coelho et al., 2012; Sabatino et al., 2020), and increasing the
production of secondary metabolites (Oliveira et al., 2015). Such fungi have shown positive effects even in the presence of pathogens (Campos et al., 2013; Cofcewicz et al., 2001), and decreasing the rate of nematode infection (Vos et al., 2012; Sá and Campos, 2020).

Studies show that AMF also bring benefits to plants even in unfavorable abiotic conditions such as tolerance to saline (Yano-Melo et al., 2003) and water stress (Cavalcante et al., 2001) and some authors even report on the benefits of AMF AMF in soils contaminated by heavy metals (Rabie, 2005; Coninx et al., 2017) such as arsenic (Xu et al., 2008).

3.4 NEMATOMIDS

Nematodes belong to the phylum Nematoda, they are animals with a vermiform body, pseudocoelomates with a generally long body. Regarding their feeding habits, nematodes are classified as free-living or parasitic, the former are found in aquatic environments and in the soil they live in water films (Bongers and Bongers, 1998).

Free-living species are classified as: Omnivores, Bacteriovores, Fungivores and Carnivores (Bongers and Bongers, 1998). Bacteriovorous and fungivorous nematodes regulate soil microbiota populations, interfering in some processes such as the decomposition of organic matter (Yeates, 2003). Some species of bacteriovores have affinity for specific types of bacteria, such as Cephalobus pseudoparvus and denitrifying bacteria (Djigal et al., 2010). Omnivores and carnivores are important in the energy flow to higher levels in the food chain (Yeates, 2003)

As for parasites, they can parasitize animals, including humans, as well as plants, causing great damage. Plant parasitic nematodes, or phytonematodes, are classified according to the type of parasitism, being ectoparasites or endoparasites, which are further subdivided into sedentary and migratory (Decraemer and Hunt, 2006).

Nematodes are considered fundamental elements in the food chain. Nematodes also participate in and control nutrient cycling (Bongers and Bongers, 1998), and can thus be used as an indicator of soil quality.

Some types of cultivation can alter nematode communities, as seen by Wardle et al. (1995) that asparagus cultivation increased the number of bacteriophages. Soil type also affects the structure of nematode communities (Cadet et al., 2003; Sá et al., 2021). In this way, these organisms are affected by soil conditions, as well as the plant species.
4 MATERIALS AND METHODS

4.1 COLLECTION LOCATION

The material was collected at the Labrunier farm, located in the city of Petrolina, Pernambuco. A collection was carried out in three areas: organic 'Sweety Globe' vine cultivation, conventional 'Sweety Globe' vine cultivation and native caatinga area close to the cultivation areas.

4.2 COLLECTED MATERIAL

It consisted of 30 soil samples collected from the rhizosphere of plants, at a depth of 0 - 30 cm, 10 samples from each area. The distance between the collection points in each area was ten meters and in a zig-zag pattern. The samples were taken to the Laboratory of Agricultural Cultures and Caatinga of the Submédio São Francisco (LACACSSF) at UPE Campus Petrolina for evaluation of AMF (spore density, mycorrhizal colonization) and nematodes.

4.3 EVALUATION OF NEMATOIDS

To identify the nematode groups, 100 ml of soil was processed using the wet sieving and centrifugation methods according to Jenkins (1964) and the nematodes obtained were quantified under a microscope and grouped according to the trophic group into: Bacteriovores, Fungivores, Omnivores and Carnivores (Bongers and Bongers 1998), or when phytonematodes identified at the genus level.

4.4 EVALUATION OF FMA

Aliquots of 50 g of soil were weighed to evaluate spore density, these were extracted by wet sieving followed by centrifugation in water and sucrose (Gerdemann and Nicolson, 1963; Jenkins 1964) and then quantified under a stereomicroscope using a channeled plate. The percentage of mycorrhizal colonization was evaluated using the quadrant intersection method (Giovannetti and Mosse, 1980), for which the roots were separated from the soil, washed in
running water to remove dirt and clarified with 10% potassium hydroxide (KOH), for a period of 22 hours, and subsequently stained with Trypan Blue (0.05%) (Phillips and Hayman, 1970).

4.5 EXPERIMENTAL DESIGN

Completely randomized type with 3 collection sites (organic cultivation, conventional cultivation and Caatinga, considered control) in 10 replications, totaling 30 experimental plots.

4.6 STATISTICAL ANALYSIS

The data were subjected to analysis of variance and the means compared by the Tukey test (P<0.05) using the Statistica program (Statsoft 1997).

5 RESULTS AND DISCUSSION

There was a significant difference between the areas in relation to the quantity of bacteriovorous, fungivorous and carnivorous nematodes, while the quantity of omnivores did not differ between the areas (Table 1). Four genera of phytonematodes were found in the studied areas (Table 2). The amount of Helicotylenchus did not differ between areas, however, the amount of Rotylenchus, Meloidogyne and Criconemela differed between the areas studied (Table 2). The density of AMF spores differed between the areas studied (Figure 1), while the percentage of mycorrhizal colonization showed no statistical difference between the areas (Figure 2).

Lower amounts of bacteriovorous nematodes were found in the area of conventionally cultivated vines compared to the Caatinga area, this reinforces the hypothesis raised by Mondino et al. (2009) that nematodes are sensitive to soil management. Villatoro (2004), working with coffee plantations (Coffea arabica L.), states that nematodes are significantly affected by the type of soil. Additionally, Pereira and Campos (2020) observed a reduction in the quantity of bacteriovorous nematodes in conventional planting of mango (Mangifera indica L.) in the Sub-middle São Francisco region, as found in the present work, reaffirming that the type of conventional management, with addition of fertilizers and chemical pesticides negatively affect bacteriovores, indirectly this indicates that the amount of bacteria is reduced in conventional planting areas.
The number of fungivores was greater in the organically cultivated vine area, differing from the other areas (Table 1), indirectly this indicates a high quantity of fungi as a food base in these areas. According to the data regarding bacteriovorous and fungivorous nematodes, apparently the food base, in the organic vine cultivation area of the present work, includes bacteria and fungi in the same proportion, since the amount of bacteriovorous and fungivorous nematodes are similar. It is worth mentioning that soil bacteria and fungi are directly involved in the decomposition of organic matter, thus they are important for keeping nutrients circulating in the food web.

The conventionally cultivated vine area showed a high number of carnivores, indicating an ecological imbalance in this area, since these nematodes are at the top of the food chain, being in smaller quantities in relation to the other trophic groups. Considering the amount of free-living nematodes, the organically cultivated vine area was similar to the Caatinga area, indicating that this area is more preserved than the conventionally cultivated area.

In general, conventional management negatively affected free-living nematodes, while organic management maintained more natural soil conditions, as it was, in relation to soil nematodes, similar to Caatinga. A similar result was found by Steinberg et al. (2022) who observed an increase in the number of free-living soil nematodes in an organically cultivated area of vines.

In the three areas collected, the number of omnivores, bacteriovores and fungivores was greater than that of carnivores (Table 1), indicating the presence of bacteria and fungi. The small number of carnivores found is due to the fact that they are at the top of the nematode food chain and therefore appear in smaller numbers. Bacteriovorous and fungivorous nematodes regulate soil microbiota populations, interfering in some processes such as the decomposition of organic matter (Yeates, 2003), a fact that explains the large quantity of these in organic areas compared to conventional ones, the deposition of organic matter increases the quantity of decomposing bacteria and fungi, thus increasing the quantity of bacteriovores and fungivores. Omnivores and carnivores are important in the energy flow to higher levels in the food chain. It is worth mentioning that nematodes are considered fundamental elements in the food chain, as they control the cycling of nutrients in the soil (Bongers and Bongers, 1998).
Table 1

*Quantity of free-living nematodes, in 100 ml of soil, from areas with organic or conventional cultivation of grapevine cv. Sweety Globe and caatinga area*

<table>
<thead>
<tr>
<th>Areas</th>
<th>Omnivores</th>
<th>Bacteriovoros</th>
<th>Fungivores</th>
<th>Carnivores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caatinga</td>
<td>312 a</td>
<td>326 a</td>
<td>30 b</td>
<td>0 b</td>
</tr>
<tr>
<td>Organic</td>
<td>316 a</td>
<td>142 ab</td>
<td>115 a</td>
<td>0 b</td>
</tr>
<tr>
<td>Conventional</td>
<td>305 a</td>
<td>134 b</td>
<td>57 b</td>
<td>51 a</td>
</tr>
</tbody>
</table>

Means followed by the same letter in the column do not differ according to the Tukey test at 5%.

Source: own authorship

In both cultivation areas, several genera of phytonematodes were found (Table 2), but not in quantities that could be harmful to cultivation. Many phytonematodes can contribute to soil maintenance, but this depends on each species (Yeates, 2003). The grapevine plants did not show any visible symptoms of meloidoginosis or other diseases caused by phytonematodes. The lower number of phytonematodes in the Caatinga area may be due to the fact that the soil in this area was in unfavorable conditions for their survival. Furthermore, monocultures are more favorable to the multiplication of phytonematodes, as well as the addition of nutrients in these areas. The organic area presented a lower amount of phytonematodes compared to the conventional area. It is known that the addition of organic matter can reduce the amount of phytonematodes (Tabarant et al., 2011).

Table 2

*Quantity of phytonematodes, in 100 ml of soil, from areas with organic or conventional cultivation of vine cv. Sweety Globe and caatinga area*

<table>
<thead>
<tr>
<th>Areas</th>
<th>Helicotylenchus</th>
<th>Rotylenchus</th>
<th>Meloidogyne</th>
<th>Criconemela</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caatinga</td>
<td>111 a</td>
<td>25 b</td>
<td>0 b</td>
<td>0 c</td>
</tr>
<tr>
<td>Organic</td>
<td>275 a</td>
<td>239 a</td>
<td>180 a</td>
<td>36 b</td>
</tr>
<tr>
<td>Conventional</td>
<td>450 a</td>
<td>305 a</td>
<td>110 a</td>
<td>168 a</td>
</tr>
</tbody>
</table>

Means followed by the same letter in the column do not differ according to the Tukey test at 5%.

Source: own authorship

The density of AMF spores in the conventional area was significantly higher than in the organic and Caatinga areas (Figure 1). In the organic area, the low quantity of spores may be due to the high number of fungivores and phytonematodes. According to Cofcewicz (2001), the presence of phytonematodes can reduce the production of AMF spores. Anjos et al (2010), studying the interaction between the AMF Fuscutata heterogama and the phytonematode Meloidogyne incognita in passion fruit (Passiflora alata), observed that the sporulation of F. heterogama is negatively affected by the presence of the nematode.
The data regarding AMF in the present work, that is, spore density and percentage of mycorrhizal colonization, were lower than those found in the literature. The quantity of spores in the organic vine area and in the Caatinga area was around 60 spores in 50 g-1 of soil, while in the conventional vine area, the quantity of spores showed an average of 140 spores in 50 g-1 of soil. A result that diverges from that found by Bezerra et al. (2022) who found around 357 spores in 50g-1 of soil collected in a vine growing area.

Mycorrhizal colonization did not differ between areas (Figure 2). The percentage of mycorrhizal colonization varied from 30%, in the Caatinga area and conventional cultivation, to 40% in the organic cultivation area. These data are lower than those found by Fors et al. (2023) who observed around 80% in the percentage of mycorrhizal colonization in grapevines.

Considering the evaluation of nematodes, the organic production area is more promising than the conventional cultivation area, as the latter negatively influences free-living nematodes, interfering with the energy flow, consequently in the edaphic conditions of the soil. In relation to AMF, they were not influenced by cultivation methods, probably the adaptive nature of these fungi allows them to adapt to the imposed environmental conditions.

In the literature it is reported that the increase in the number of organisms in organically managed soil is greater and more diverse than in other types of management, including in vines, as observed by Karimi et al. (2020) who state that soil microorganisms are three to four times higher in organic viticulture than in conventional viticulture. Doring et al. (2019) observed that in 17 of the 24 studies with organic vine production there was an increase in soil biodiversity, at all trophic levels. Such results were also obtained in the present work, in relation to nematofauna, reinforcing that the use of organic management is beneficial for the soil and consequently will be beneficial for the plant, especially when thinking about the long term, as this type of management is natural and sustainable, not polluting the soil, as normally occurs in conventional planting.
**Figure 1**

*Density of spores of arbuscular mycorrhizal fungi, in 50 g of soil, from organic or conventional cultivation of grapevine cv. Sweety Globe and caatinga area. Means followed by the same letter do not differ according to the Tukey test at 5%*

*Source: own authorship*

**6 CONCLUSIONS**

Conventional cultivation of vines cv. Sweety Globe, negatively affects free-living nematodes in the soil;

The cultivation of vines cv. Sweety Globe, favors the multiplication of phytonematodes, regardless of the type of cultivation;
The type of vine cultivation cv. Sweety Globe, does not affect mycorrhizal colonization; Conventional cultivation of vines cv. Sweety Globe stimulates AMF spore production.
REFERENCES


Nematofauna and Arbuscular Mycorrhizal Fungi in Organic and Conventional Vine (Vitis vinifera L.) Plantations in the Submedium São Francisco Valley


