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FTIR-ATR para identificação de plantas de *Psidium guajava* infestado com *Meloidogyne enterolobii*

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Abstract

The *Meloidogyne enterolobii* Yang and Eisenback nematode represents one of the most devastating pests in guava cultivation in Venezuela and the world. The diagnosis of this parasite requires specialized knowledge and very laborious procedures. The objective of this research was to identify the infrared spectra of guava plants, in the nursery phase, infested with *M. enterolobii* using Fourier-transform infrared spectroscopy coupled to attenuated total reflectance (FTIR-ATR). Leaves from healthy and infested plants were taken 60 days after nematode inoculation and analyzed in a FTIR-ATR spectrometer. The main spectral bands corresponding to the chemical compounds (lipids, proteins and carbohydrates) produced by plant metabolism as a result of nematode infestation were characterized. These results represent the starting point to determine the potential of this rapid and non-destructive technique for the early diagnosis of plants infested by the "guava root-knot nematode".

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Resumen

El nematodo *Meloidogyne enterolobii* Yang y Eisenback representa una de la plagas más devastadora en el cultivo del guayabo en Venezuela y el mundo. Para el diagnóstico de este parásito se requieren conocimientos especializados y procedimientos muy laboriosos. El objetivo de esta investigación consistió en identificar los espectros infrarrojos de plantas de guayabo, en fase de vivero, infestadas con *M. enterolobii* empleando la espectroscopía infrarroja con transformada de Fourier acoplada a reflectancia total atenuada (FTIR-ATR). Se tomaron hojas de plantas sanas e infestadas, 60 días luego de la inoculación del nematodo y se analizaron en un espectrómetro FTIR-ATR. Se caracterizaron las principales bandas espectrales, correspondientes a los compuestos químicos de las regiones de lípidos, proteínas y carbohidratos producidos por el metabolismo de las plantas a consecuencia de la infestación por el nematodo. Estos resultados representan el punto de partida para determinar el potencial de esta técnica rápida y no destructiva para el diagnóstico temprano de plantas infestadas por el "nematodo agallador del guayabo".

Palabras clave: Espectroscopía infrarroja transformada de Fourier, guayabo, nematodo agallador del guayabo, diagnóstico.

Resumo

O nematóide *Meloidogyne enterolobii* Yang e Eisenback representa uma das pragas mais devastadoras no cultivo de goiabeira na Venezuela e no mundo. O diagnóstico deste parasita requer conhecimento especializado e procedimentos muito laboriosos. O objetivo desta pesquisa foi identificar os espectros de infravermelho de plantas de goiabeira, em fase de viveiro, infestadas com *M. enterolobii* utilizando espectroscopia de infravermelho com transformada de Fourier acoplada à refletância total atenuada (FTIR-ATR). Folhas de plantas sadias e infestadas foram retiradas 60 dias após a inoculação do nematoide e analisadas em espectrômetro FTIR-ATR. Foram caracterizadas as principais bandas espectrais correspondentes aos compostos químicos (lipídios, proteínas e carboidratos) produzidos pelo metabolismo da planta como resultado da infestação de nematoides. Esses resultados representam o ponto de partida para determinar o potencial desta técnica rápida e não destrutiva para o diagnóstico precoce de plantas infestadas pelo "nematóide das galhas da goiaba".

Palabras-chave: Espectroscopia de infravermelho com transformada de Fourier, goiabeira, nematoide das galhas da goiaba, diagnóstico.

Introduction

Meloidogyne enterolobii Yang and Eisenback represents one of the most important root-knot nematodes in the world due to its aggressive pathogenicity, wide geographical distribution, and host plant range (Castagnone-Sereno, 2012). In *Psidium guajava* L. this nematode is the cause of "guava dieback", representing one of the most devastating pests in Venezuela (Crozzoli & Casassa, 1998; Perichi & Crozzoli, 2010) and the world (Ashokkumar & Poornima, 2019).

In this sense, early detection of this pest is the key to its control, and it is mainly limited by its characteristic of being a parasite of the guava tree, therefore the diagnostic process in the analysis of soil and root samples requires time, and the acute expertise of specialists in the area.

Since 1990, the characterization of various biological tissues began using Fourier-transform infrared spectroscopy (FTIR), based on the vibration and rotation of molecules in the infrared region of the electromagnetic spectrum, allowing the different components of a sample to be identified (Baker *et al*., 2014). The main advantage of this technique is the ease and speed with which data can be obtained and the precision of the analysis of spectral bands in the characterization of the different tissue components (lipids, proteins, and carbohydrates); however, its application in studies of plant diseases and pathogens is scarce and recent (Gandolfo *et al*., 2016; Huleihel *et al*., 2018; San Blas *et al*., 2011).

Therefore, in this research it was established to identify the infrared spectra present in guava plants, in the nursery phase, healthy and infested with *M. enterolobii*, using Fourier-transform infrared spectroscopy coupled to attenuated total reflectance (FTIR-ATR).

Materials and methods

Location of the research

It was carried out in the shade-house of The Socialist Center for Fruit and Beekeeping Research and Development (CESID Fruit and Beekeeping-CORPOZULIA) 10°49'46.6'' LN, 71°46'29.2'' LO), located in the Mara municipality of Zulia state, Venezuela.

Plant material

A seedbed was prepared with a sterilized substrate composed of goat manure and sand in a 1:2 ratio and seeds of ''Criolla Roja'' type guava, susceptible to *Meloidogyne* spp. were sown (Crozzoli & Casassa, 1998). Thirty days after germination they were transplanted into plastic containers (500 cm^3).

Obtaining the inoculum

A population of *M. enterolobii* (Perichi & Crozzoli, 2010) from guava trees grown in the CESID-CORPOZULIA field was used. Females with egg masses were extracted from the roots and placed in Petri dishes, and a hole (1.5 cm deep) was opened in the substrate of 20 guava plants (3 months old), and an egg mass (containing 800 to 3,000 eggs) was carefully placed in the substrate. The plants were placed on plateaus in the greenhouse (28 ± 2 °C, 70% relative humidity, and 12 h of light) and watered by hand every other day. These plants served as an inoculum source for the investigation.

It should be noted that 45 days after inoculation, five plants were randomly selected and 20 individual females were extracted from the roots and sent to Dr. Regina Carneiro, a specialist researcher in the identification of phytoparasitic nematode species, assigned to the Center for Genetic Resources and Biotechnology, belonging to the Brazilian Agricultural Research Company (EMBRAPA), located in the city of Brasília DF, Brazil, with whom we obtained confirmation of the identification indicated by Perichi and Crozzolli (2010) as *M. enterolobii*.

Experimental phase

Forty 30-day-old guava plants were inoculated with 10 g of roots from the inoculum source plants (described above) and ten were used as a control treatment (healthy plants). These were placed in the greenhouse. Sixty days after inoculation, the second pair of leaves (Butler *et al*., 2015) was taken from each healthy and infested plant and transferred to the laboratory where they were dried at room temperature (25 °C) for 24 h and ground in an agate mortar to a fine powder.

Acquisition of spectra

Each powder sample was placed in a Shimadzu© Prestige model 21 FTIR spectrophotometer with a DLATGS detector, to which was attached a MIRacle™ brand attenuated total reflectance (ATR) accessory with a 6 mm tri-reflecting diamond/ZnSe crystal with high pressure (200 psi) in the wave number range from 4,000 to 500 cm^{-1}, with a spectral resolution of 4 cm^{-1}, scanning 150 (measurements) per sample, following the procedure described by San Blas *et al*. (2011). The resulting spectra were coded and analyzed. The experiment was performed in triplicate.

Spectral data analysis

A completely randomized experimental design was used with two treatments: 10 healthy plants (PgS) and 40 infested plants (PgMe). Each plant represented the experimental unit. Prior to the statistical analyses, the ATR correction function was applied to the spectra obtained to eliminate the absorption of atmospheric water vapor and CO_2. Also, the baseline was corrected and the absorbance data were normalized (between 0 and 1). This processing was performed using the IRSolution software included in the spectrometer.

Then, the spectral region corresponding to the mid-infrared (4,000 and 500 cm^{-1}) was selected, the section with the greatest utility and precision in the analysis of plants (Gandolfo *et al*., 2016) and in the identification of entomopathogenic nematodes (San Blas *et al*., 2011). Finally, the characterization of bands was carried out from a visual differentiation of the spectra of healthy plants and those infested with the nematode.

Results and discussion

Characterization of healthy and infested guava plants with *Meloidogyne enterolobii* by FTIR-ATR infrared spectroscopy

Figure 1 shows the absorption bands obtained in the region of the infrared spectrum between 4,000 and 500 cm^{-1} that identify the vibrational modes of the different functional groups that constitute the biomolecules of the PgS and PgMe plants analyzed.

The characterization of the absorption bands in PgS and PgMe leaves is presented in table 1. The hydroxyl group O-H bond stretching or N-H group stretching vibration bands corresponding to the lipid region were assigned to ~3273 and ~3229 cm^{-1}. Bands equivalent to this region have been identified in various plant tissues (leaves, stems, and seeds, among others) (Kumar *et al*., 2016; Türker-Kaya & Huck, 2017).

Table 1. Characterization of absorption bands from infrared spectra with attenuated total reflectance in leaves of healthy guava plants (PgS) and infested (PgMe) with *Meloidogyne enterolobii,* in the region between 4000 and 500 cm^{-1}.

PgS (cm^{-1})	PgMe (cm^{-1})	Region and Vibration Mode
		Lipid region.
3273	3229	Stretching of the O-H bond of the hydroxyl group or N-H stretching.
2920	2922	Asymmetric C-H stretching of methylene groups (CH_2) of acyl lipid chains.
2857	2857	Symmetric C-H stretching of methylene groups (CH_2) of acyl lipid chains.
		Carbohydrate region.
1728	1724	Stretching of the C-H bond and C=O double bond of saturated esters of cellulose, hemicellulose, pectin, lignin, cutin.
		Protein region.
1695	1694	Amide I: β-turns and β-folded leaves, antiparallel and β-folds.
1616	1622	
1545	1549	Amide II: N-H and C-N bending stretching of the protein chain.
1518	1516	
		Mixed region: proteins and carbohydrates.
1445	1449	C-C: aromatic compounds -phenols-.
1371	1368	Symmetric bending in CH_2 and CH_3 (polysaccharides: cellulose, hemicellulose).
		Protein region.
1314	1298	Amide III: C-N and N-H stretching of proteins (symmetric bending in CH_3).
1234	1231	C-O stretching: lignin and xylan.
		Carbohydrate region.
1169	1161	C-O-C symmetric stretching: cutin.
1099	1092	Asymmetric stretching of pectic substances.
1038	1034	O-H stretching and C-O bending of the C-O-H group of cell wall carbohydrates (cellulose, hemicellulose, pectins, lignin).
		Carbohydrate region.
878	---	Stretching in CH.
824	---	CH_2 group of fatty acids.

For the following bands at ~2920, ~2922, and ~2857 cm^{-1} (table 1), intense asymmetric and symmetric C-H (-CH_2-) stretching vibrations were observed, respectively, corresponding to acyl chains of lipids with a contribution of proteins, carbohydrates, and nucleic acids. Similarly, these bands were also characterized by Kumar *et al.*

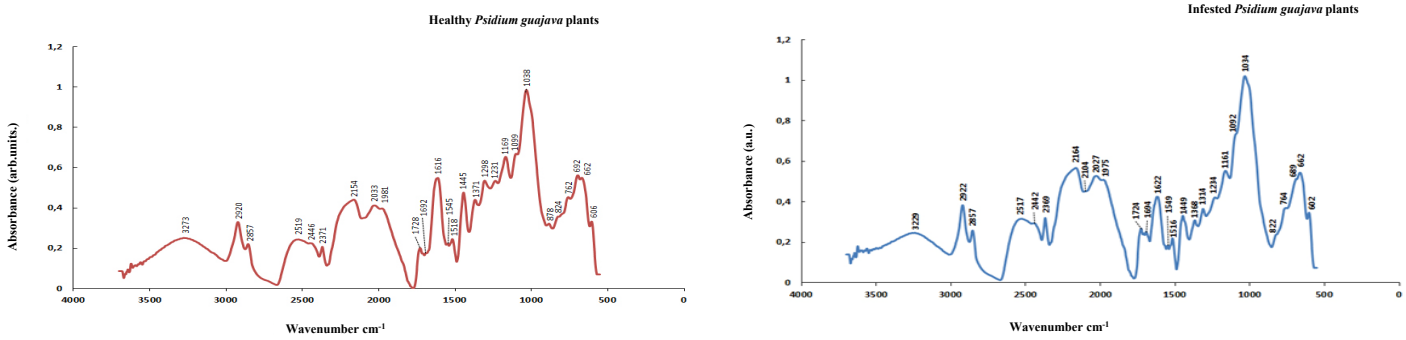

Figure 1. Average infrared spectra in leaves of healthy guava plants (●) and plants infested (●) with *Meloidogyne enterolobii*, by means of infrared spectroscopy (region between 4000 and 500 cm^{-1}).

(2016) and Türker-Kaya and Huck (2017) in plant tissue analysis. Eloh *et al*. (2016) in physiological studies found a reduction in fatty acids and lipids (glycerol, myristic acid, and palmitic acid) in plants infested with nematodes.

On the other hand, the bands ~1728 and ~1724 cm^{-1} (figure 1 and table 1) were attributed to the stretching vibration of the C-H bond and C=O double bond of saturated esters of cellulose, hemicellulose, pectin, lignin, and cutin, corresponding to the carbohydrate region. Equivalent bands have been assigned in several studies of the chemical constitution of leaves of different plant species (Liu *et al*., 2014; Suresh *et al*., 2016). Gandolfo *et al*. (2016) point to this region in studies of the "dieback" of Japanese pine (*Larix kaempferi* (Lamb.) Carr.) caused by the *Phytophthora ramorum* Werres fungus.

The following identified bands belong to the protein region, specifically, ~1695 and ~1694 cm^{-1} (table 1) and the bands ~1622 and ~1616 cm^{-1} (table 1) assigned to the Amide I region (proteins) in β-fold vibrations, β-turns, β-folded leaves, antiparallel. Likewise, the bands ~1549 and ~1545 cm^{-1} and around ~1518 and ~1516 cm^{-1} (table 1) which correspond to the Amide II region (proteins) in N-H and C-N bending stretching vibrations of the main protein chain. These protein regions were reported by Heredia *et al.* (2014), Jiang *et al.* (2015), Kim *et al*. (2016), Kumar *et al.* (2016), Türker-Kaya and Huck (2017) in various studies of plant tissues (leaves, flowers, fruits, among others).

Subsequently, a mixed region (1500-1200 cm^{-1}), which contains proteins and carbohydrates, was characterized. Starting from bands ~1449 and ~1445 cm^{-1} to ~1234 and ~1231 cm^{-1}, in PgS and PgMe respectively (table 1). The bands ~1449 and ~1445 cm^{-1} assigned to aromatic compounds (phenols) and ~1371 and ~1368 cm^{-1} of symmetric bending vibration in CH_3 (polysaccharides: cellulose, hemicellulose). Likewise, the bands ~1314 and ~1298 cm^{-1}, corresponding to the Amide III region (proteins), with C-N and N-H stretching vibrations of proteins and the bands at ~1234 and ~1231 cm^{-1} correspond to C-O stretching vibrational modes (lignin and xylan).

Studies carried out by San Blas *et al.* (2011) report these protein bands (amide I and amide III) in the differentiation of entomopathogenic nematode species (*Steinernema glaseri* Gerdin and Bedding and *Heterorhabditis indica* Karunakar and David). Gandolfo *et al*. (2016) and Hawkins *et al.* (2010), found protein bands with absorption peaks with lower intensity (content decrease) in leaves of citrus plants (*Citrus* sp.) infested with the *Candidatus liberibacter* bacterium, the cause of the Huanglongbing or "Yellow Dragon" disease, in contrast to healthy plants. Likewise, Huleihel *et al*. (2018) determined this region for the identification of different genera of phytopathogenic fungi (*Rhizoctonia, Colletotrichum, Verticillium,* and *Fusarium)*. Also, this region has been characterized in the analysis of leaves of "Navel" orange plants (*Citrus sinensis* L.) with and without boron deficiency (Liu *et al.,* 2014), in the variation of biochemical components (proteins and carbohydrates) in leaves of different plant species: peanut (*Arachis hypogaea* L.) (Suresh *et al*., 2016), artichoke (*Cynara cardunculus* var. scolymus L.) (Kim *et al*., 2016). Also, in the characterization of proteins (amide III region) in soybean, quinoa, and rice (Córdoba *et al*., 2020).

Similar results to this research have been reported in physiological studies on roots of aubergine (*Solanum melogena* L.), papaya (*Carica papaya* L.), jasmine (*Jasminum* sp.) (Farahat *et al*., 2013), okra (*Abelmoschus esculentus* (L.) Moench) (Sharma *et al*., 2018), where a decrease in protein content was determined after infestation caused by *M. incognita.* Similarly, Lobna *et al*. (2017), determined biochemical alterations in the amount of protein in tomato roots induced by *M. javanica-Fusarium oxysporum* interaction. Eloh *et al.* (2016) and Sharma and Sharma (2017) found a reduction in protein content in leaves of tomato plants infested with *M. incognita*. These low protein levels occur during the later phases of infection and suggest that the multiplying nematodes continuously extract significant amounts of nutrients from the giant cells causing a decrease in nutrient and water uptake and consequently stunted growth.

In this spectral region, the band interval ~1449 and ~1445 cm^{-1} stands out, which represents bands of phenolic compounds that are characterized by increasing in plants with the advance of the infestation caused by nematodes, as pointed out by Pérez-Pérez *et al.* (2014), who carried out chemical analysis and determined an increase in the content of phenolic compounds in guava leaves grown in a field infested with *M. enterolobii.* Similarly, Rahman *et al*. (2018) using high-performance liquid chromatography reported the presence of phenolic compounds (catechin, epicatechin, and ellagic acid) in extracts (powder) of guava leaves. Similarly, Kesba and El-Beltagi (2012), determined an increase in the content of total phenols in rootstocks of grapevine (*Vitis vinifera* L.) plants infested with *M. incognita* and *Rotylenchulus reniformis* Linford and Oliveira. Also, Lobna *et al.* (2017), correlated variations in total phenol content with co-infection caused by *M. javanica* and *F. oxysporum* in tomato roots. Sharma and Sharma (2017) determined an increase (66 %) of phenols in tomato plants attacked by *M. incognita.* In this regard, Dhakshinamoorthy *et al.* (2014) showed that phenols and lignin play an important role in the defense mechanisms of *Musa* sp. against *Radopholus similis* Thorne infestation, highlighting the increase of the biosynthesis and/or accumulation of secondary metabolites such as phenolic phytoalexins at the sites of infection by this nematode.

After, the spectral region corresponding to carbohydrates (1200-900 cm^{-1}) (polysaccharide fingerprints) is presented (figure 1 and table 1). The absorption bands ~1169 and ~1161 cm^{-1} correspond to C-O-C (cutin) symmetric stretching vibrations. At ~1099 and ~1092, they were assigned to asymmetric stretching of pectic substances. Also, bands ~1038 and ~1034 cm^{-1} were identified as O-H stretching and C-O bending of the C-O-H group of carbohydrates and cell wall polysaccharides (cellulose, hemicellulose, lignin, pectins, cutin). This carbohydrate spectral region has allowed discriminating healthy *Pinus* spp. plants from those infested with the nematode *Bursaphelenchus xylophilus* Steiner and Buhrer (Gandolfo *et al*., 2016). San Blas *et al.* (2011) were able to differentiate entopathogenic nematode species (*S. glaseri* and *H. indica*) in this region. Also, Gandolfo *et al*. (2016) and Hawkins *et al*. (2010) established carbohydrate bands for differentiation between healthy and infested citrus plants with *C. liberibacter*. Also, corresponding to the above, physiological studies by Arce-Leal *et al*. (2019) determined that as this bacterial disease progresses in Mexican lemon (*Citrus aurantifolia* Christm (Swingle) plants, the starch content increases in relation to healthy plants, associated with phloem plugging by photoassimilates.

Likewise, in the analysis of leaves with FTIR, Buitrago *et al.* (2018) established the differences between 19 plant species by determining cellulose and lignin. Topală *et al.* (2017) pointed to

this region for the diagnosis of viral diseases in grapevine (*Vitis* spp.) cultivars. Also, Huleihel *et al*. (2018) reported it, for the identification of several genera of phytopathogenic fungi (*Rhizoctonia, Colletotrichum, Verticillium,* and *Fusarium*). It has also been used for the characterization of leaf tissue in tomato (Butler *et al*., 2015), artichoke (Kim *et al*., 2016), peanut (Suresh *et al*., 2016), and in studies of sugars in mango pulp (Olale *et al.,* 2017), in the composition (cellulose, β-glucopyranose) of sugarcane bagasse (Zara *et al*., 2017), in the composition of pea seeds, *Pisum sativum* L. (Jiang *et al*., 2015), in the compounds of various plants (Heredia *et al*., 2014).

Studies of biochemical alterations in tomato roots caused by *M. javanica-F. oxysporum* interaction indicate an increase in the concentration of carbohydrates in infested plants (Lobna *et al*., 2017). Similarly, Eloh *et al*. (2016) in metabolic studies on leaves of tomato plants infested with *M. incognita* determined, through gas chromatography and mass spectrometry analysis, an increase in carbohydrate content.

Finally, a region with bands below ~900 cm^{-1} was identified (figure 1 and table 1) which exhibit a very low (weak) spectral resolution, making it difficult to characterize, however, the ~878 cm^{-1} band can be identified with a stretching vibrational mode in CH of carbohydrates (Butler *et al*., 2015; Heredia *et al*., 2014; Kumar *et al*., 2016, Türker-Kaya & Huck, 2017). Also, the ~824 cm^{-1} band has been pointed out with vibrational modes of oscillation of the CH_2 group of fatty acids in analyses of microorganisms (Neugebauer *et al*., 2007).

Conclusions

The application of Fourier-transform infrared spectroscopy (FTIR-ATR) allowed to identify the bands present in the different regions of the infrared spectrum (4,000 and 500 cm^{-1}) (lipids, proteins, and carbohydrates) that constitute the biomolecules of guava plants in the nursery phase, healthy and infested with the *M. enterolobi* nematode*,* establishing the basis for generating a simple, fast, non-destructive and efficient diagnostic protocol. The next phase of this research includes the analysis of the second derivative of the infrared spectrum in the "fingerprint region" (1800 and 900 cm-1) that will reveal the biochemical changes (specific bands of phenols, proteins, and carbohydrates) in the physiology of guava plants to discriminate between healthy and infested guava plants, in order to design phytosanitary strategies for the management of these plant parasites.

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Literature cited

Arce-Leal, A., Leyva-López, N., Santos-Cervantes, M., Rodríguez-Negrete, E., Méndez-Lozano, J., Manzanilla-Ramírez, M. & Perea-Flores, M. (2019). Cambios en el metabolismo de carbohidratos asociados a la infección por *Candidatus liberibacter asiaticus* en limón mexicano (*Citrus aurantifolia*). *Agrociencia 53*(4):593-604. https://dialnet.unirioja.es/servlet/articulo?codigo=7015501

Ashokkumar, N. & Poornima, K. (2019). Occurrence and distribution of root knot nematode, *Meloidogyne enterolobii* in guava (*Psidium guajava* L.) in Tamil Nadu. *Journal of Pharmacognosy and Phytochemistry 8*(2), 1922-1924. https://www.phytojournal.com/archives/2019/vol8issue2/PartAF/8-2-400-354.pdf

Baker, M., Trevisan, J., Bassan, P., Bhargava, R., Butler, H., Dorling, K., Fielden, P., Fogarty, S., Fullwood, N., Heys, K., Hughes, C., Lasch, P., Martin-Hirsch, P., Obinaju, B., Sockalingum, G., Sulé-Suso, J., Strong, R., Walsh, M., Wood, B. , Gardner, P. & Martin, F. (2014). Using Fourier transform IR spectroscopy to analyze biological materials. *Nature Protocols 9*, 1771-1791. https://europepmc.org/articles/pmc4480339/bin/nihms700198-supplement-suppl_file.pdf

Buitrago, M., Skidmore, A., Groen, T. & Hecker, C. (2018). Connecting infrared spectra with plant traits to identify species. *Journal of photogrammetry and remote sensing 139*, 183-200. https://www.sciencedirect.com/science/article/abs/pii/S0924271618300789

Butler, H., McAinsh, M., Adams, S. & Martin, F. (2015). Application of vibrational spectroscopy techniques to non-destructively monitor plant health and development. *Analytical Methods 7*, 4059-4070. https://pubs.rsc.org/en/content/articlehtml/2015/ay/c5ay00377f

Castagnone-Serrano, P. (2012). *Meloidogyne enterolobii* (= *M. mayaguensis*): profile of an emerging, highly pathogenic, rootknot nematode species. *Nematology 14*,133–138. https://brill.com/view/journals/nemy/14/2/article-p133_1.xml

Córdoba, D., Parra, A., Solanilla, J., Hoyos, J. & Roa, D. (2020). Study of the antioxidant and structural properties of quinoa, rice, and soy protein isolates. *SYLWAN 164*(5), 353-368. https://www.researchgate.net/profile/Diego-Fernando-Roa/publication/341580513_Study_of_the_antioxidant_and_structural_properties_of_quinoa_rice_and_soy_protein_isolates/links/615a46554a82eb7cb5f49d87/Study-of-the-antioxidant-and-structural-properties-of-quinoa-rice-and-soy-protein-isolates.pdf

Crozzoli, R. & Casassa, A. (1998). Especies de *Meloidogyne* asociadas al cultivo del guayabo (*Psidium guajava* L.) en el Municipio Mara, Estado Zulia, Venezuela. *Revista de la Facultad de Agronomía (LUZ) 15*(1), 107-108. https://produccioncientificaluz.org/index.php/agronomia/article/view/26171/26796

Dhakshinamoorthy, S., Mariama, K., Elsen, A. & De Waele, D. (2014). Phenols and lignin are involved in the defence response of banana (*Musa*) plants to *Radopholus similis* infection. *Nematology 16*, 565-576. https://brill.com/view/journals/nemy/16/5/article-p565_4.xml

Eloh, K., Sasanelli, N., Maxia, A. & Caboni, P. (2016). Untargeted metabolomics of tomato plants after root-knot nematode infestation. *Journal of Agricultural and Food Chemistry 64*, 5963-5968. https://pubs.acs.org/doi/abs/10.1021/acs.jafc.6b02181

Farahat, A., Al-Sayed, A. & Mahfoud, N. (2013). Growth response and chances in chemical composition in some host plants caused by infection with three nematode species. *Egyptian Journal Agronematology 12*, 139-158. https://www.researchgate.net/journal/Egyptian-Journal-of-Agronematology-

Gandolfo, D., Mortimer, H., Woodhall, J. & Boonham, N. (2016). Fourier Transform Infra-Red Spectroscopy using an Attenuated Total Reflection probe to distinguish between Japanese larch, pine and citrus plants in healthy and diseased states. *Spectrochimica Acta 163*, 181-188. https://www.sciencedirect.com/science/article/abs/pii/S138614251630124X

Hawkins, S., Park, B., Poole, G., Gottwald, T., Windham, W. & Lawrence, K. (2010). Detection of citrus Huanglongbing by Fourier transform infrared attenuated total reflection. *Applied Spectroscopy 64*(1), 100-103. https://journals.sagepub.com/doi/abs/10.1366/000370210790572043

Heredia, J., Benítez, J., Domínguez, E., Bayer, I., Cingolani, R., Athanassiou, A. & Heredia, A. (2014). Infrared and Raman spectroscopic features of plant cuticles: a review. *Frontiers Plant Science 5*(305), 1-14. https://www.researchgate.net/profile/Jose-Heredia-Guerrero/publication/263364161_Heredia-Guerrero_et_al_2014/links/00b4953aab793ddee9000000/Heredia-Guerrero-et-al-2014.pdf

Huleihel, M., Shufan, E., Tsror, L., Sharaha, U., Lapidot, I., Mordechai, S. & Salman, A. (2018). Differentiation of mixed soil-borne fungi in genus level using infrared spectroscopy and multivariate analysis. *Journal of Photochemistry and Photobiology B: Biology 180*, 155-165. https://www.sciencedirect.com/science/article/abs/pii/S1011134417307443

Jiang, Y, Lahlali, R., Karunakaran, C., Kumar, S., Davis, A. & Bueckert, R. (2015). Seed set, pollenmorphology and pollen surface composition response to heat stress in field pea. *Plant, cell and environment 38*(11), 2387-2397. https://onlinelibrary.wiley.com/doi/epdf/10.1111/pce.12589

Kesba, H. & El-Beltagi, H. (2012). Biochemical changes in grape rootstocks resulted from humic acid treatments in relation to nematode infection. *Asian Pacific Journal of Tropical Biomedicine 2*(4), 287-293. https://www.sciencedirect.com/science/article/abs/pii/S2221169112600240

Kim, C., Seong, K., Bin, J., Lim, C., Moon, D. & Song, S. (2016). Establishment of discrimination system using multivariate analysis of FT-IR spectroscopy data from different species of artichoke (*Cynara cardunculus* var. scolymus L.). *Korean Journal of Horticultural Science and Technology 34*, 324-330. https://koreascience.kr/article/JAKO201615964634202.page

Kumar, S., Lahlali, R., Liu, X. & Karunakaran, C. (2016). Infrared spectroscopy combined with imaging a new developing analytical tool in health and plant science. *Applied Spectroscopy Reviews 51*:466-483. https://www.tandfonline.com/doi/abs/10.1080/05704928.2016.1157808

Liu, G., Dong, X., Liu, L., Wu, L., Peng, S. & Jiang, C. (2014). Boron deficiency is correlated with changes in cell wall structure thatlead to growth defects in the leaves of navel orange plants. *Scientia Horticulturae 176*:54–62. https://www.sciencedirect.com/science/article/abs/pii/S030442381400363X

Lobna, H., Aymen, E., Hajer, R., M'Hamdi-Boughalleb, N. & Horrigue-Raouani, N. (2017). Biochemical and plant nutrient alterations induced by *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. radicis lycopersici co-infection on tomato cultivars with differing level of resistance to *M. javanica*. *European Journal of Plant Pathology 148*, 463–472. https://www.proquest.com/openview/041cb8bd630bf68af041bd9718ac64ed/1?pq-origsite=gscholar&cbl=55385

Neugebauer, U., Schmid, U., Baumann, K., Ziebuhr, W., Kozitskaya, S., Deckert, V., Schmitt, M. & Popp, J. (2007). Towards a detailed understanding of bacterial metabolism-spectroscopic characterization of *Staphylococcus* Epidermidis. *ChemPhysChem 8*(1), 124-137. https://chemistry-europe.onlinelibrary.wiley.com/doi/abs/10.1002/cphc.200600507

Olale, K., Walyambillah, W., Mohammed, S., Sila, A. & Shepherd, K. (2017). Application of DRIFT-FTIR spectroscopy for quantitative prediction of simple sugars in two local and two Floridian mango (*Mangifera indica* L.) cultivars in Kenya. *Journal of Analytical Science and Technology 8*, 1:1-13. https://www.proquest.com/openview/1ab436008280b9e4a34cf8a653f66191/1?pq-origsite=gscholar&cbl=2034725

Pérez-Pérez, E., Ettiene, G., Marín, M., Casassa-Padrón, A., Silva, N., J. Raga, C. González, L. Sandoval & D. Medina. (2014). Determinación de fenoles y flavonoides totales en hojas de guayabo (*Psidium guajava* L.). *Revista de la Facultad de Agronomía (LUZ) 31*, 60-77. https://produccioncientificaluz.org/index.php/agronomia/article/view/27149

Perichi, G. & Crozzoli, R. (2010). Morfología, morfométría y hospedantes diferenciales de poblaciones de *Meloidogyne* de los estados Aragua y Zulia, Venezuela. *Fitopatología Venezolana 23*(1), 6-15.https://www.researchgate.net/profile/guillermo-perichi/publication/48221047_morfologia_morfometria_y_hospedantes_diferenciales_de_poblaciones_de_meloidogyne_de_los_estados_aragua_y_zulia_venezuela/links/555676b508ae980ca60c8773/morfologia-morfometria-y-hospedantes-diferenciales-de-poblaciones-de-meloidogyne-de-los-estados-aragua-y-zulia-venezuela.pdf

Rahman, M., Zaman, S., Mamun, F., Gias, Z., Alam, M., Ulla, A., Hossain, H. & Alam, A. (2018). Phenolic content analysis in *Psidium guajava* leaves powder by HPLC-DAD system and *in vivo* renoprotective and antioxidant activities in fludrocortisone acetate-induced rats. *Journal of Food Biochemistry*. e12687. https://onlinelibrary.wiley.com/doi/abs/10.1111/jfbc.12687

San-Blas, E., Guerra, M., Portillo, E., Esteves, I., Cubillán, N. & Alvarado, Y. (2011). ATR/FTIR characterization of *Steinernema glaseri* and *Heterorhabditis indica*. *Vibrational Spectroscopy 57*, 220-228. https://www.sciencedirect.com/science/article/abs/pii/S0924203111000968

Sharma, I. & Sharma, A. 2017. Physiological and biochemical changes in tomato cultivar PT-3 with dual inoculation of mycorrhiza and PGPR against root-knot nematode. *Symbiosis 71*, 75-183. https://www.infona.pl/resource/bwmeta1.element.springer-doi-10_1007-S13199-016-0423-X

Sharma, N., Khajuria, Y., Sharma, J., Tripathi, D., Chauhan, D., Singh V., Kumar, V. & Singh, V. (2018). Microscopic, elemental and molecular spectroscopic investigations of root-knot nematode infested okra plant roots. *Vacuum 158*, 126-135.https://www.sciencedirect.com/science/article/abs/pii/S0042207X18311795

Suresh, S., Karthikeyan, S. & Jayamoorthy, K. (2016). FTIR and multivariate analysis to study the effect of bulk and nano copper oxide on peanut plant leaves. *Journal of Science: Advanced Materials and Devices 1*(3), 343- 350. https://www.sciencedirect.com/science/article/pii/S2468217916301125

Topală, C., Tătaru, L., Buciumeanu, E. & Guţă, I. (2017). FTIR spectra of grapevines (*Vitis vinifera* L.) in the presence of virus infections. *Acta Horticulturae 1188*, 313-318. https://www.cabdirect.org/cabdirect/abstract/20183271505

Türker-Kaya, S. & Huck C. (2017). A review of mid-infrared and near-infrared imaging: Principles, concepts and applications in plant tissue analysis. *Molecules 22*(168), 2-20. https://pdfs.semanticscholar.org/93e1/dadca96bf0aa93a5139e4aad8ca904648d07.pdf

Zara, J., Yegres, F., Vargas, N., Cubillan, L., Navas, P. & Márquez, M. (2017). Empleo de la espectroscopía infrarroja (FT-IR-ATR) como herramienta para la caracterización del bagazo de caña proveniente de la Sierra Falconiana. *Química Viva 16*(3), 17-24. https://www.redalyc.org/pdf/863/86354619003.pdf