Study of Molecular and Elemental Changes in Nematode-infested Roots in Papaya Plant Using FTIR, LIBS and WDXRF Spectroscopy

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ABSTRACT: Papaya is a tropical fruit of the *Carica papaya* plant and grown all over the world. Root knot nematodes are one of the major problems in papaya fruit crop production and causes maximum yield loss every year by slowly killing the plant. In this article, we study healthy as well as nematode-infested papaya plants at the atomic and molecular levels to obtain information about the causes and changes occurring in the plant after infestation. Microscopic observations were made to check the presence of pathogens in the plant. Elemental profiling of healthy and infested papaya plants was assessed by using two advanced spectroscopic techniques, namely laser-induced breakdown spectroscopy (LIBS) and wavelength dispersive X-ray fluorescence spectroscopy (WDXRF). The structural changes in the complex bio compounds, such as starch, protein, lipids, fatty acids, and carbohydrates, were assessed by Fourier transform infrared spectroscopy (FTIR). The elemental and molecular profiling results of the infested vs. the healthy papaya plants were compared to establish why the plants die.

INTRODUCTION

Plant parasitic nematodes are greatly responsible for the major destruction of agricultural crops. The annual global loss due to this infestation for most of the economically valued agricultural crops, such as vegetables, fruits, and ornamental plants, is estimated to be 14%, which amounts to an economic loss of about \$80 billion worldwide.¹ Among all of the parasitic nematodes, root knot nematodes (*Meloidogyne spp.*) are considered the leading problem for agricultural crops. Depending on the species of the nematode, type of crops and geographical region, the estimated crop loss was about 5-43% (reported in 2014).² The estimated crop loss (reported in 2017) due to the infestation of nematodes, particularly

in India, was about Rs. 242.1 billion.³ This infestation of the root knot nematode degrades the quality and production of agricultural foods and ultimately leads to a global loss to the agricultural industry.⁴ Recently Lima *et al.* reviewed the negative effect of plant parasitic nematodes that are silently impacting the overall soybean yield and also presented a picture of symptoms, biology and control methods used for these nematodes species.⁵

Papaya trees are susceptible to various diseases, including root rot, powdery mildew, papaya rings pot, poty virus, damping off,⁶ but of the nematode diseases the two genera, namely *M. incognita* and *M. Javanica*, are reported to be the most economically detrimental.⁷ Since nematode-infested plants are more prone to

attack by fungi, this becomes even more complex in association with other microbes. Heavy destruction from the infestation of the root knot nematode has been well reported by various countries.⁸ The affected papaya plants develop galls or knots, especially on the roots, resulting in poor and stunted growth due to the loss of water uptake capacity, including other nutrients. This disease becomes a major constraint in the successful cultivation of papaya plants and reduces the annual yield and income for thousands of already poor farmers.

With the advancement of technology, many nematicides are being used as a remedy to control the number of nematodes and their effect. However, the use of nematicides or other related chemicals are not beneficial and cause toxicity to the soil and the environment.⁹ In order to alleviate these barriers towards achieving sustainability in agriculture, many researchers from the agronomy, soil and plant sciences are trying to develop new strategies to support and maintain the increasing global demand for food.

Many researchers are turning towards spectroscopic analytical methods to obtain a clearer understanding about the changes that might occur in agricultural food stuffs due to the invasion of pathogens. Soil sample testing and plant tissue analysis by advanced analytical techniques are the only way left for efficiently working on these problems. Sharma *et al.* used spectroscopic methods for the analysis of okra root samples and to study their chemical imbalances created due to nematode infection.¹⁰ Proper analysis of the elemental and molecular changes due to nematode infection provides information how to tackle plant nutrition disorder and thus, leads to implementing the necessary actions to prevent future plant loss due to nematodes and other microbes.

The same process as utilized in this article with the spectroscopic techniques was also applied to FTIR spectroscopy and LIB spectroscopy (LIBS) for assessing the molecular changes and elemental changes that might take place with the infestation of nematodes. In addition, the reproducibility of the LIBS data was checked by standard X-ray fluorescence spectrometry (XRF), and the results of both methods were compared. Therefore, an attempt is made in this study to provide inexpensive control measures for farmers to eliminate this disease.

EXPERIMENTAL

Collection of papaya plant roots (healthy and nematode-infested). Healthy and nematode-infested papaya plant samples were collected from a field site in Jammu from the Jammu and Kashmir states in India. The nematode-infested papaya plant roots were washed to remove the dirt and soil, and then dried in contamination-free area in sunlight. Fig. 1 shows the presence of knots on the roots. After drying, the roots were separated from the plant, chopped and finely ground using mortar and pestle.

Fig. 1 Snapshot of root knot infested papaya plant roots.

FTIR, WDXRF and LIBS spectroscopic techniques. The Perkin Elmer RXZ Fourier transform infrared spectrophotometer (PerkinElmer, Inc., USA) was used to record the FTIR spectra of the ground papaya root samples in the transmittance mode, in the spectral region of 4000–400 cm^{-1} , with a spectral resolution of 1 cm^{-1} . The FTIR spectra were recorded in a very simple manner as only a few milligrams of the papaya samples were mixed with KBr to make the KBr pellets. The pellets were prepared by pressing the mixture under the pressure of 8 tons for 5 min.

The model RT100-EC instrument (ASI, USA) was used to read and record the LIBS spectra of the papaya plant samples (in pressed pellet form). A Q-switched Nd: YAG laser beam, operating at 1064 nm, was focused on plant root sample surfaces by an objective lens (5 $\times$ magnification, 35 mm working distance). The wavelength, pulse duration, pulse energy, and repetition rate were 1064 nm, 10 ns, 17 mJ/pulse, and 10 Hz, respectively. The detector used was a multichannel charge-coupled device (CCD) spectrometer. The laser spot size on the surface of root samples was 100 μm in diameter. The number of laser shots accumulated for a single LIBS spectrum was 50 shots. For one sample, 20 line scans were recorded and 20 line scans were performed for each sample pellet. Finally, the 20 spectra were averaged to predict the LIBS results. These conditions provided good signal-to-noise ratios. The optical emission from the laser-produced plasma was then collected using two lenses through the top quartz window and is finally sent to the CCD equipped spectrometer with ~ 0.1 nm spectral resolution and wavelength coverage between 187 nm and 887 nm. The CCD detection gate with 1.05 ms width was delayed from the laser pulse by 0.5 μs to minimize continuum background emission and spectral line broadening.

The quantitative measurements of the elemental constituents of the papaya plant samples were performed using a model S8 TIGERWDXRF spectrometer (Bruker, Germany). In this WDXRF spectrometer, an end-window X-ray tube with ^{45}Rh anode is adjusted to a voltage of 60 kV and a current of 170 mA, correlating to a maximum power supply of 4 kW to generate the primary beam.

To find the more accurate and reproducible results, the plant samples were analyzed three times. To measure the concentration of the trace and heavy elements present in the papaya plant samples, S8 TIGERWDXRF spectrometer uses its own standard-less semi-quantitative *Spectra^{plus}* software (*Quant Express*) package with reliability and reproducibility.

RESULTS AND DISCUSSION

Identification of nematodes. Root knot nematodes, *Meloidogyne spp.*, on the papaya plant roots are the most significant pest problems found in several fields of Jammu region. They cause severe plant stunting, chlorosis, and immense root galling. Using morphological characteristics, we have extracted females, males, and juveniles by using the Cobb's sieving and decanting method for further species identification. The root knot nematode was examined and identified by cutting perineal patterns of the females. For this study, the procedure was adopted as described by Goodey et al. (1963).¹¹ First, the galled roots were separated from the affected papaya roots, cleaned in water, immersed in a beaker containing boiling 0.1% cotton blue, and then left standing overnight. The female nematodes were teased out from the galls and transferred to a drop of lactophenol placed on a glass slide. The posterior portion of the females was cut off very carefully, and the remaining parts were cleaned. The perineal pattern of the adult females was trimmed off and mounted on the slide for examination. For each examination, 10 slides were prepared which contains the perineal pattern, head shape, stylet length, and length of the juvenile nematodes. We identified the species according to the methods reported by Eisenback et al., while comparing the perineal region's characteristics.¹² Thus, we have observed that the root knot nematode species which affected the papaya plant roots was *Meloidogyne incognita*.

FTIR spectroscopy of papaya plant roots. The molecular study of the compounds present in any plant body can be easily ascertained by using FTIR spectroscopy. It is mainly used for the identification of different biomolecules that were absorbed in the IR range. We employed this method in order to obtain the spectral changes that might have occurred due to the infestation of the root knot nematodes in the diseased papaya plant samples. For this, we recorded the FTIR spectra of both a healthy and a diseased papaya plant in the spectral range of 4000-500 cm^{-1} with a spectral resolution of about 4 cm^{-1} (Fig. 2). The recorded spectrum was first converted into absorbance by subtracting the base line; then it was normalized to identify the presence of lipids, starch, protein and other complex molecules in both the diseased and the healthy papaya plant samples. The plotted spectra of the healthy and diseased papaya plant samples were analyzed to check for the presence of protein, carbohydrates, fats, lipids and moisture content. Both spectra were compared to determine the spectral changes.

Fig. 2 FTIR Spectra of healthy papaya plant (black line) and nematode-infested papaya plant (red line) in the range of 4000-500 cm^{-1} .

Typically, the absorption bands in the FTIR spectrum reflect the molecular composition. The corresponding intensities showed the constituents present in the papaya plant samples. Since it is difficult to eliminate all water content from the samples, the absorption bands due to water were also observed in the IR spectra.¹³ The peak at 3382 cm^{-1} is due to OH stretching and can be assigned to the moisture content in the healthy and diseased papaya plant samples.¹⁴ Water absorbs strongly in the mid-IR range due to the presence of H-bending and OH-stretching, but its presence sometimes is hindered by the appearance of other OH containing compounds, such as alcohol, phenols, and hydrogen peroxide.¹⁵

The protein content in the papaya plant can be realized by the appearance of two absorption peaks of the amide I group and amide II group. The absorption bands of the amide I group at 1650 cm^{-1} and the amide II group at 1545 cm^{-1} show proof of the protein content in the healthy and diseased papaya plant samples, respectively. The amide I group, which appeared as a peak at 1650 cm^{-1} in the IR spectrum, basically arises due to the C=O stretching of the peptide bonds of the protein molecules in the papaya plant samples.¹⁴ The peak for the amide II group at 1545 cm^{-1} originated due to the coupling of the two N-H bending and C-N stretching vibrations and, consequently, give the amide II group as the backbone of the protein in the healthy and diseased papaya plant sample.¹⁴ According to the FTIR spectra, the presence of amide II group is observed only in the nematode-infested papaya plant samples, which clearly indicates that the overall protein quantity increased, while the amide II group is absent in the healthy papaya plant samples. The increase in protein content can be considered as one of the consequences of root knot nematode infestation and other microbes, all of which affect the natural quality of the papaya plant. Starch and carbohydrates are the major constituents of the papaya plant. Amylopectin and amylose are the main carbohydrates and their presence is reflected in the absorption bands of the FTIR spectrum. The most frequent region for the

analysis of starch and carbohydrates in the IR spectral region is the anomeric region of 950–750 cm^{-1} , in which the characteristic bands of 'a' and 'b' conformers or the pyranoid and furanoid ring vibrations of the monosaccharide and polysaccharide are feasibly discriminated.¹⁰ In our work, the absorption bands that are responsible for the vibration modes of the amylopectin and amylase components of the carbohydrates appeared in the spectral region of 1500–800 cm^{-1} , whereas region 2800–3000 cm^{-1} is responsible for the C-H stretching and region 3000–3600 cm^{-1} is attributed to O-H and N-H stretching. The characteristics of lipids are shown by the corresponding C-H stretching in the region of 2800–3000 cm^{-1} . On the other hand, the absorbance band at 1739 cm^{-1} also appeared due to the stretching vibrations of $\nu(\text{C}=\text{O})$ of the ester group of lipids and fatty acids. In our results, the peaks at 2928 cm^{-1} , 2855 cm^{-1} and 1731 cm^{-1} are responsible for the ester group in the lipids and fatty acids.¹⁰ The absorption bands at 1459 cm^{-1} can be assigned to the methyl and methylene groups of the lipids and proteins in the papaya plant.¹⁰ The presence of carbohydrates in the papaya plant is confirmed by the bands at 1154 cm^{-1} , 1103 cm^{-1} , 1082 cm^{-1} and 1034 cm^{-1} , thereby indicating the influence of nematode infection due to starch and glycogen. The decrease in the starch content as observed in the FTIR spectrum of the infected papaya plant sample (in comparison to the healthy papaya plant sample) showed that all of the starch in the plant sample is attacked by the nematode and fungus.

From the FTIR spectrum obtained for the healthy and infected papaya plant samples, it is clear that the major components of the plants, such as carbohydrates, proteins, lipids and fats, were greatly affected by the infestation of nematodes and fungi. The decrease in carbohydrates in the infected papaya plant samples is due to an increase in amylase activity during a fungus and nematode attack. It also adversely reduces the starch content of the plant by hydrolyzing it into simple sugar, such as maltose and glucose, while the protein content increases. These proteins are stimulated in the plant body in order to activate the defense mechanism against the parasites coming in during the invasion of pathogens and produces reactive oxygen species and antimicrobial compounds.¹⁶ These carbohydrate and protein patterns were also confirmed by Tayal and Agarwal who observed an increase in activity of amylase in *M. incognita* infected *Solanum melongena*.¹⁷ The same decreasing pattern of carbohydrates and an increase in proteins was also observed by Farahat *et al.* in their investigations with *M. incognita* nematode-infected root samples of eggplant, papaya and jasmine.¹⁸ The present FTIR study shows that the changes occurring in the complex biomolecules of carbohydrates, proteins, lipids, and fatty acids in the diseased papaya plant samples are due to nematode infestation and fungus.

LIBS of papaya plant roots. Fig. 3a shows the LIBS spectra of healthy and diseased papaya plant samples in the full spectral range, whereas Fig. 3(b–f) shows the parts of the full LIBS spectra, thereby revealing the presence of various major and trace elements

present in the papaya plant samples. The LIBS spectra of the healthy and diseased papaya plant samples shows the presence of Mg, Si, Al, Ca, Na, K, N, O, C, Na, Zn, Fe, Ti, Cr, Mn, Cu and Cl, however, their intensity varies due to the nematodes in the root knots. The detection and identification of the elements in the LIBS spectra is totally based on the presence of strong and persistent lines which were carefully resolved by using the NIST database profile.¹⁹ Fig. 3b shows one of the parts of full LIBS spectra of the healthy and diseased papaya plant roots in the spectral region 279–291 nm, and correspondingly indicating the presence of Mg and Si with the appearance of sharp resonance emission peaks at 279.55 nm, 280.27 nm, 285.21 nm and 288.15 nm. In Fig. 3c, the resonance lines of Ca and Al can easily be identified by the emission peaks observed at 393.38 nm, 396.84 nm and 394.40 nm, 396.15 nm, respectively. The element characteristic line intensity gives the profile of the relative concentrations of the elements, so the intensities of these elements can be correlated with their concentrations.²⁰ In Fig. 3(b, c), the elements Mg, Si, Ca and Al follow the same trend of $2>1$, i.e., indicating that the diseased papaya plant sample accumulated more Mg, Si, Ca and Al than the healthy plant. The presence of K in two papaya plant samples can be easily seen at the corresponding emission peaks at 766.48 nm and 769.89 nm. The intensities of K and Mn in the diseased papaya plant samples show a decreasing pattern due to nematode infestation. The presence of the Na and N lines in Fig. 3d and 3f can be easily identified in the wavelength range from 554–640 nm & 725–766 nm. It can clearly be seen that the intensity of the Na lines is higher in the healthy papaya plant samples than the diseased plant, whereas the content of N is higher in the diseased papaya plant samples. Besides these elements, we also found some weak emission lines for Fe, Ti, Zn, S, Cl, Cr and Cu for all of the plant samples with different concentrations. It has been concluded that the mineral elements Ca, Mg, Fe, Al, Si, and N follow the pattern $2>1$, whereas the elements P, K, Na, Cu, Zn, Mn and Cl follow the pattern $1>2$. The reason for an increase in Ca, Mg, Si, Al and Fe and a decrease in K, P, Mn, Cl, Na, Zn and Cu in the papaya plant samples can be considered due to nematode infestation. Due to various effects like self-absorption, signal uncertainties, matrix effects, and shot-to-shot variations, there is a higher chance of uncertainties that can question the accuracy of the observed LIBS data. In order to validate our results or to know the reproducibility of the LIBS data, we employed another elemental technique, namely wavelength dispersive X-ray fluorescence spectrometry (WDXRF), not only for comparing the information regarding the changes in elemental composition of the papaya plants, but also the quantitative details of the elements, i.e. how much the element increased or decreased in association with the nematode infestation on the papaya plant roots.

WDXRF of papaya plant roots. The WDXRF methodology was employed for obtaining the elemental spectra of healthy and diseased papaya plants in order to observe what elemental changes

Fig. 3 LIBS Spectra of healthy papaya plant (black line) and nematode-infested papaya plant (red line) in the wavelength range of 187–887 nm (a) and in different ranges and thereby shows the presence of the elements Mg, Si, Ca, Al, Na, N and K (b–f).

might occur due to the nematode's infestation. Fig. 4(a–f) shows the overlapped WDXRF spectra of the healthy and diseased papaya plant samples with different energy ranges, thereby indicating the presence of the elements Al, Ca, Cl, Cu, Mg, Mn, K,

Si, S, Cr, Zn, Na, P, Fe, Ni, and Rb in both plant samples but in different concentrations. The quantification of these elements detected in the WDXRF spectra was performed using Quant Express software provided in the S8 Tiger WDXRF spectrometer

Fig. 4 WDXRF spectra of healthy and diseased papaya plant samples in different energy ranges.

(Bruker Corporation, Germany). The X-ray energies of all of the elements detected in the papaya plant samples are summarized in Table 1.

After analyzing the WDXRF spectra of the healthy and diseased papaya plants, it was observed that the concentrations of the elements in the healthy papaya plant changed after nematode

infestation. It was observed that the production of the mechanical barriers, induction of inhibitory compounds and activation of defense mechanisms are related to the continuous availability of essential nutrients in the plants.²¹ The mineral elements of Ca and Mg showed that their concentration values jumped from lower to higher values. The concentration of Ca jumped from 24,600 ppm

Table 1. XRF Energies of the Elements Detected in the WDXRF Spectra of Papaya Plant Roots

Elements	Energy (KeV)	
	K_{α_1}	K_{β_1}
Oxygen (O)	0.525	----
Magnesium (Mg)	1.254	----
Aluminum (Al)	1.484	1.578
Silicon (Si)	1.740	1.836
Phosphorus (P)	2.015	2.140
Sulfur (S)	2.310	2.465
Chlorine (Cl)	2.625	2.820
Potassium (K)	3.318	3.594
Calcium (Ca)	3.696	4.013
Chromium (Cr)	5.415	----
Manganese (Mn)	5.900	6.49
Iron (Fe)	6.419	7.057
Nickel (Ni)	7.488	----
Copper (Cu)	8.046	8.904
Zinc (Zn)	8.637	9.567
Rubidium (Rb)	13.428	----
Zirconium (Zr)	15.775	----

Table 2. Concentration of Elements in Papaya Plant Samples Were Measured by WDXRF (ppm)

Elements	Healthy papaya plant root		Nematode infested papaya plant root	
	Conc.	± SD**	Conc.	± SD
Ca	24600	1230	31400	1570
K	13300	665	10700	535
Cl	1700	85	1400	70
Mg	3900	195	4600	230
Si	2300	115	5300	265
P	2300	115	2000	100
S	2000	100	2000	100
Na	1300	65	700	35
Al	800	40	2700	135
Fe	800	40	1700	85
Cr	92	4.6	63	3.15
Mn	76	3.80	48	2.4
Zn	77	3.85	57	2.85
Cu	29	1.45	21	1.05
Ti	33	1.65	100	5
Sr	25	1.25	19	0.95
Ni	9	0.45	13	0.65
Ru	19	0.15	ND	ND
Pd	13	0.65	ND	ND
Zr	ND*	ND	1	0.05

*ND: Not detected; **SD: Standard deviation

in the healthy plant to 31,400 ppm in the diseased plant, whereas the concentration of Mg jumped from 3900 ppm in the healthy plant and to 4600 ppm in the diseased plant. Calcium and magnesium are the major nutrients in the papaya plant and play an important role in defending the plant by maintaining the outer cell walls during the invasion of pathogens. It has also been reported that Ca helps in regulating the flow of sugar and essential amino acids from the cells to the intercellular spaces in the plant tissues,

which further helps in maintaining the balance between sugar and amino acids to distract the attention of pathogens.²² Silicon is another element which is well-known for its importance in activating the various defense mechanisms and antioxidant compounds in maintaining the plant defense system against pathogens.²² The concentration of Si increased and jumped from 2300 in a healthy to 5300 in a nematode-infested papaya plant. The same increasing pattern of Ca, Mg and Si was observed by Sharma *et al.* in nematode-infested okra plant roots.¹⁰ The increase in concentration of Ca, Mg and Si can be correlated with the activation of the defense system during the invasion of pathogens. The concentrations of some other elements, such as Fe, Al and Ti, also increased in the papaya plant with nematode infestation. Al and Cr are not considered essential elements for plants, but due to their association in soil they somehow manage to enter the plant body at very low concentrations.

The mineral elements of potassium and phosphorus are considered essential macroelements for papaya plants and play many roles such as enzyme activation, protein synthesis, photosynthesis, stomatal movements, energy transfer, phloem transport, cation-anion balance, and biotic stress resistance.²² After analyzing the WDXRF spectra, we observed that the concentration of K and P decreased in relation to nematodes in the papaya plant. It was reported that potassium in combination with phosphorus promotes thicker cuticles and cell walls and produces mechanical barriers to infection by invading pathogens.²³ From the WDXRF results, it was observed that the concentration level of potassium dropped from 13,300 ppm in healthy papaya plants to 10,700 ppm in nematode-infested papaya plants, whereas the concentration level of phosphorus dropped from 2300 ppm in the healthy to 2000 ppm in nematode-infested papaya plants. There are some elements, such as Na, Zn, Mn and Cu, which also show a decreasing pattern after the invasion of nematodes. The same decreasing pattern of the elements Na, Zn, Mn and Cu was reported by Sharma *et al.* in nematode-infested okra plant roots.¹⁰ Some elements, such as Zr and Mo, were observed only in diseased plant samples and are totally absent in healthy papaya plant samples. Plant disease is the result of the interactions of various factors, *i.e.* the host plant's resistance to pathogens, its relation to the availability of good nutrients, the pathogens and their capacity to develop and invade the host tissues.²³ It is also reported that the pathogens after invading the host plant developed strategies to bypass host defenses and deprived them of certain elements, while the host plant developed ways to limit the movement of pathogens by increasing the concentration of some minerals in the plant.²⁴ As discussed above, the decrease or increase in the concentration of some of the elements can be considered as one of the major consequences due to nematode infestation and hamper the life of the papaya plant.

Comparison of LIBS and WDXRF results. The validation of this study was performed by using two elemental techniques,

Fig. 5 Comparison of the concentrations of elements present in papaya plant samples (healthy and nematode-infested papaya plant) measured by using LIBS and WDXRF methods.

Table 3. Peak Area Intensity (Concentration) in Papaya Plant Root Samples Were Measured by LIBS

Elements	Healthy Papaya Plant			Nematode-infested Papaya Plant		
	Peak area intensity	± SD*	± RSD**	Peak area intensity	± SD	± RSD
Ca (393.36 nm)	37.595	4.990	0.13	43.076	3.547	0.08
Mg (279.55 nm)	2.511	0.232	0.09	3.104	0.194	0.06
K (766.48 nm)	16.941	0.828	0.05	13.823	0.790	0.06
Si (288.15 nm)	0.099	0.019	0.19	0.265	0.032	0.12
Na (588.99 nm)	7.739	0.383	0.05	5.702	0.314	0.06
Fe (238.40 nm)	0.101	0.014	0.14	0.323	0.051	0.16

*SD: Standard deviation; **RSD: Relative standard deviation

LIBS and WDXRF, for assessing the elemental changes in healthy and nematode-infested papaya plant samples. The element characteristic line intensity is proportional to the concentration in the sample when there is no self-absorption in the LIBS method.²⁰ We also evaluated all plasma parameters to verify the conditions of the existence of stoichiometric ablation, optical thickness of the plasma and local thermodynamic equilibrium, which further confirms that no self-absorption occurs in the plasma of the LIBS system. So, the peak area intensity obtained with LIBS was calculated for Ca (393.36 nm), Mg (279.55 nm), K (766.48 nm), Si (288.15 nm), Fe (238.40 nm) and Na (588.99 nm), which will be considered with the concentration values. Since LIBS and WDXRF are different in nature, we cannot compare their concentration values directly. Instead, we focused on the patterns that each and every element followed in the healthy and the nematode-infested papaya plant samples in each of the elemental techniques and which is also visible in the bar diagrams of Fig. 5. The mineral elements Ca, Mg and Si follow the pattern 2>1 in the LIBS method and can easily be seen in Table 2. It shows the peak area intensity values of the papaya plant samples along with their relative standard deviations. The same pattern is used in Table 3 for the WDXRF method with respect to the elemental

concentrations in the papaya plant samples. From the WDXRF and LIBS concentration values listed in Tables 2 and 3, it can be observed that K and Na follow the pattern 1>2 in the LIBS spectra of papaya plant samples and is well matched with the same pattern for the WDXRF spectra. Fig. 5 shows the bar diagrams in which the concentrations of the major elements Ca, Mg, Si, K, Fe and Na are compared with both the LIBS and WDXRF methods. Since the same pattern for the LIBS and WDXRF analysis of papaya plant samples was followed, it can be stated that LIBS and WDXRF are the most efficient tools for the qualitative and quantitative study of mineral elements in healthy as well as diseased papaya plant samples.

CONCLUSIONS

Spectroscopic analytical methods are nowadays being used by various researchers in the field of agriculture and food sciences in order to ensure good quality food and to monitor the changes that might take place in the food crops with regard to invading pathogens. In this article, we have successfully applied the spectroscopic techniques of FTIR, LIBS and WDXRF for assessing the molecular and elemental changes that might occur in

the papaya plant with the appearance of gall nematodes. This article is very helpful in understanding disease interactions with specific mineral nutrients, their effect on the host plant and the environment, which may further help to improve disease control, enhance yield productivity and improve crop quality.

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Notes

The authors declare no competing financial interest.

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