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Spectroscopical differences among soil samples of selected susceptible and resistant varieties of banana in relation to wilt disease

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Abstract: Banana is an important cash crop cultivated in several states of India, including Tamil Nadu. Wilt disease caused by the fungal pathogen is a severe disease of banana resulting in great economic loss to the farmers. Although the pathogen occurs in the soil and in suckers, the visible symptoms may be seen only after several months. In the meantime, for the development of disease, the physico-chemical factors of the soil along with the atmospheric factors play an important role. So it is an important step to maintain the unfavourable soil condition for the pathogen by analysing the soil samples. In the present study, spectroscopical analysis has been done in order to know the difference between the soil of healthy and diseased plants of five susceptible varieties and five resistant varieties of banana from various localities of Tirunelveli district, Tamil Nadu. The results of macronutrients (NPK) obtained by classical quantitative methods, have been confirmed by IR spectroscopic analysis. Thus the IR spectroscopic analysis of soil samples clearly shows the difference in absorption spectrum between soil samples of susceptible (diseased and healthy) and resistant varieties of banana. Such spectroscopic difference is mainly due to the difference in concentration of macronutrients (NPK). Thus it is concluded that, when compared to tedious-classical methods of soil analysis, the simple and quick spectroscopic analysis of the soil will be more useful to identify the soil which is more conducive for the development of wilt disease in Banana.

Keywords: Spectroscopic analysis, soil, Banana wilt disease

INTRODUCTION

India has first position in the world in banana production. Banana has occupied a top position in India's booming fruit industry with an annual production of 13.5 mt from an area of 4.0 lakh ha. But India has a vision for increasing the production to 25 mt by the end of 2020 AD and has been addressed systematically during the last decade. But still there is a long way to go to achieve target yield potential, which is being threatened from time to time by various biotic and abiotic stresses associated with banana production [1].

Panama disease, also known as *Fusarium* wilt of banana (*Musa* spp.), is one of the most notorious of all plant diseases [2]. Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* continues to pose threat among commercial Indian cultivars. Twenty-two percent of total banana production remains at the mercy of this pathogen [1]. Since *Fusarium* is a soil borne fungus, the physico-chemical factors of the soil play an important role in the development of the wilt disease in banana. Hence, soil fertility management should best be combined with pest management in order to increase the yield of banana. [3] found that imbalance in P/Zn and K/Mg ratios led to *Fusarium* wilt in a cultivar that was supposed to be resistant. Likewise, [4] found that Zn-deficient bananas were more affected by *Fusarium* wilt than non-deficient plants. Wortmann and Kaizzi (1998) [5] have also mentioned that in the long term, there is a greater need for P and K fertilizers, than for N fertilizers.

Disease-suppressive soils are found in several different locations [6]. Although disease suppression has been associated with chemical and physical edaphic factors, reasons for the phenomenon differ in various locations. For example, a close relationship between suppression and clay (montmorillonoid type) soils was found in tropical America, but in the Canary Islands suppression was associated with host mineral nutrition. Unfortunately, no reports have been made on the transfer of suppression to a disease-conducive soil [7]

Due to the above reasons, evaluation of soil fertility is now becoming a routine work for soil management and crop production. However, laboratory-analysis based determination of soil properties is time and cost consuming, which is not suitable for precision agriculture. Infrared spectroscopy (IR) appears as an alternative and fast technique to measure soil characteristics. A major breakthrough in these studies has been the use of visible-near infrared spectroscopy to develop

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quantitative calibrations for rapid characterization of soil nutrients and physical properties. In the present study, it has been aimed to compare the characteristics of soils from disease prone areas and disease free areas. In order to know the difference in physico-chemical characteristics between conducive soil and suppressive soils, five different susceptible varieties (both healthy and diseased) along with five different resistant varieties from different localities of Tirunelveli district, Tamilnadu have been selected and compared. In order to know the validity of spectroscopical method, the results have also been compared with the quantitative analysis of macro and micronutrients of soil samples by classical methods.

MATERIALS AND METHODS

The soil samples of healthy and diseased plants of five susceptible varieties and five resistant varieties of banana were collected from various localities of Tirunelveli District, Tamilnadu during the months of November and December, 2009. The plants were about 6 months old. The soil samples were collected from the study area from the depth of 30cm as per standard procedure of IARI (Indian Agricultural Research Institute) New Delhi [8]. The sampling sites were randomized to avoid biasing results.

Soil characteristics like pH, EC, available Nitrogen, available Phosphorus, available Potash and percentage of organic carbon were determined by standard methods [9]. The soil samples were subjected to chemical analysis for estimating Fe, Mn, Cu and Zn through AAS (Linday & Norwill 1978)

One of the important applications of FTIR spectroscopic study is the diagnostic value to establish the variation in organic content in plant or soil samples. In such an attempt, either a frequency shift or the variation of the intensity of the characteristic bands can be used (Ramaswamy et al. 1980).

For this study the soil samples were oven dried at 110ºC individually and ground to a fine powder. FTIR spectra were recorded using KBr pellet technique. The spectra of all powdered and pelletised samples were recorded under identical condition in the 4000-400cm-1 region using NICOLET-AVATAR 360 FTIR spectrometer.

RESULTS AND DISCUSSION

The pH of the soils from healthy plants of susceptible varieties varies from 6.71 to 7.93 and it is from 7.82 to 8.22 in soils of resistant varieties. In contrast, the pH range is comparatively low (6.0 – 7.2) in soils from diseased plants of susceptible varieties (Table 1& 2). Thus the present study generally shows that the soils with pH below 7.0 are conducive to fusarium wilt and the soils with above pH 7.0 are resistant to fusarium wilt. Soils with higher degree (0.39 – 0.58) of Electrical Conductivity are more conducive to fusarium wilt as observed in the soils of diseased plants of susceptible varieties. Lower Electrical Conductivity has been observed in soils of healthy plants of susceptible and resistant varieties. i.e 0.13 – 0.29, 0.11 – 0.21dsm^{-1} (Table 1 & 2,).

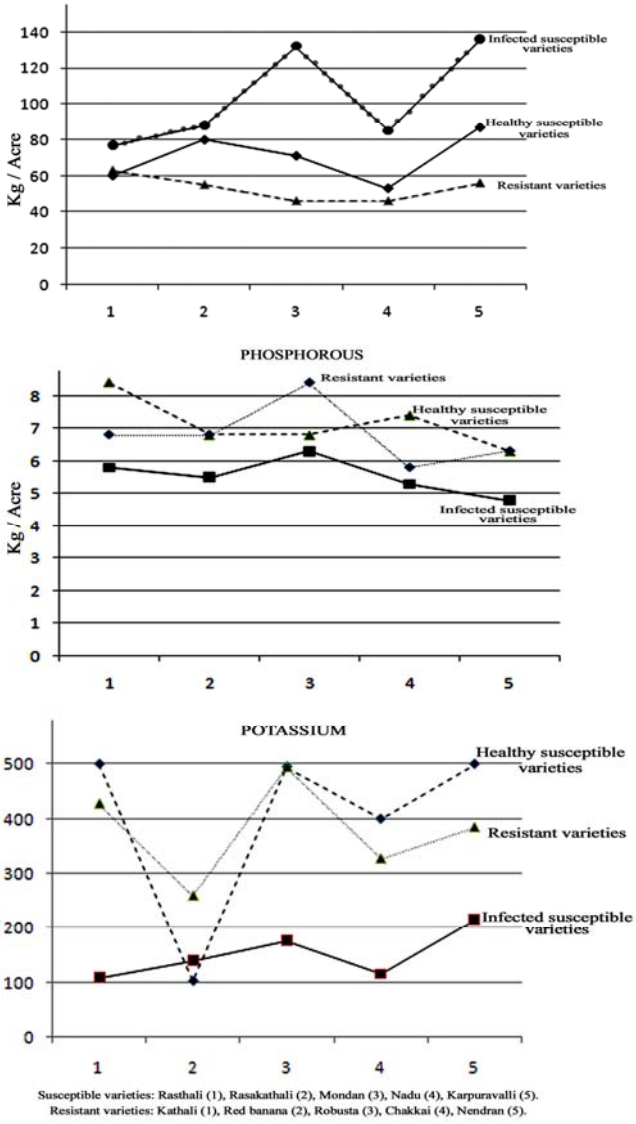
Table 1. Physico-chemical parameters of soil samples of susceptible (healthy / Diseased) and resistant varieties of banana.

Varieties	pH	EC Dsm^{-1}	Macronutrients (Kg/Acre)			Trace Minerals (ppm)			
			Nitrogen	Phosphorous	Potassium	Iron	Copper	Manganese	Zinc
Susceptible varieties (Healthy and diseased)									
Rasthali (Healthy)	7.9	0.18	60	6.8	500	25.19	0.216	11.74	0.498
Rasthali (Diseased)	6.0	0.4	77	5.8	108	2.523	0.237	5.054	0.254
Rasakathali (Healthy)	7.93	0.13	80	6.8	103	05.023	0.013	08.815	0.545
Rasakathali (Diseased)	6.2	0.39	88	5.5	140	0.752	0.582	4.61	0.307
Mondan (Healthy)	6.71	0.27	71	8.4	495	06.065	0.643	6.959	0.349
Mondan (Diseased)	6.0	0.58	132	6.3	175	2.065	0.44	3.088	0.275
Nadu (Healthy)	6.98	0.29	53	5.8	400	08.545	.....	7.308	0.289
Nadu (Diseased)	6.4	0.53	85	5.3	115	2.878	0.257	4.769	0.227
Karpuravalli (Healthy)	7.98	0.18	87	6.3	500	5.033	.....	12.98	0.367
Karpuravalli (Diseased)	7.2	0.44	136	4.8	215	2.523	0.338	8.351	0.349
Resistant varieties									
Kathali	8.22	0.21	63	8.4	428	24.84	0.115	11.52	0.879
Red banana	7.84	0.11	55	6.8	260	06.065	0.257	06.005	0.911
Robasta	7.82	0.17	46	6.8	495	09.608	0.806	03.310	0.296
Chakkai	8.0	0.18	46	7.4	328	12.44	0.44	07.22	0.445
Nendran	8.09	0.2	56	6.3	385	5.357	0.257	14.25	0.37

Table 2. Minimum and maximum values of physico-chemical parameters of soil samples of susceptible (healthy/diseased) and resistant varieties of banana

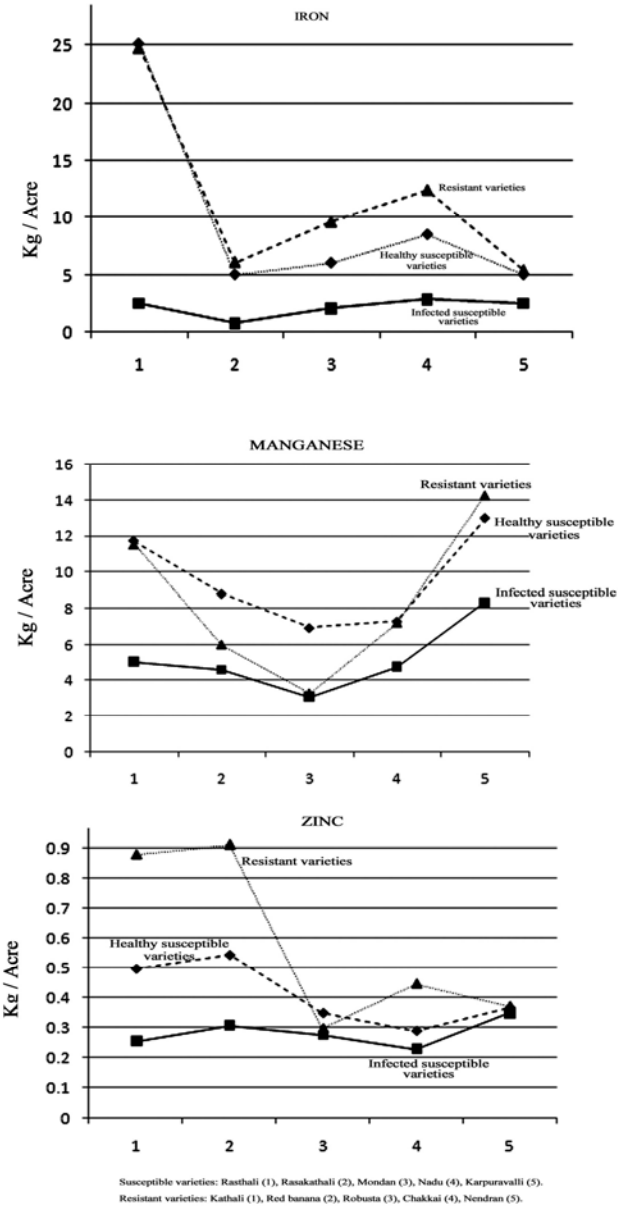
Physico-chemical characters	Susceptible varieties		Resistant varieties
	Healthy	Diseased	
pH	6.71 – 7.93	6.0 – 7.2 (Low)	7.82 – 8.22
EC	0.13 – 0.29	0.39 – 0.58 (High)	0.11 – 0.21
Nitrogen	53 - 87	77 – 136 (High)	46 - 63
Phosphorous	5.8 – 8.4	4.8 – 6.3 (Low)	6.3 – 8.4
Potassium	103 -500	108 – 215 (Low)	260 - 495
Iron	5.023 – 25.19	0.75 – 2.878 (Low)	6.065 – 24.84
Copper	0.013 – 0.643	0.237- 0.582	0.115 – 0.806
Manganese	6.959 – 12.98	3.088 – 8.351 (Low)	3.31 – 14.25
Zinc	0.289 – 0.545	0.227 – 0.349 (Low)	0.296 – 0.911

Fig. 1. Macronutrients



Susceptible varieties: Rasthali (1), Rasakathali (2), Mondan (3), Nadu (4), Karpuravalli (5).
Resistant varieties: Kathali (1), Red banana (2), Robusta (3), Chakkai (4), Nendran (5).

Fig. 2. Micronutrients



Susceptible varieties: Rasthali (1), Rasakathali (2), Mondan (3), Nadu (4), Karpuravalli (5).
Resistant varieties: Kathali (1), Red banana (2), Robusta (3), Chakkai (4), Nendran (5).

When the soil samples of healthy plants of susceptible and resistant varieties are compared with the soil samples of diseased plants of susceptible varieties of banana, there is much variation in macronutrients such as Nitrogen, Phosphorous and Potassium (Table 1 & 2, Fig. 1). Nitrogen is comparatively higher in amount (77 – 87 kg/ acre) in soil samples of diseased plants of susceptible varieties when compared with the soil samples of healthy plants of susceptible (53 – 87 Kg /Acre) and resistant varieties (46 – 63 Kg / Acre). Reverse trend is seen in the amount of Phosphorous and Potassium. Thus low amounts of Phosphorous (4.8 – 6.3 Kg / Acre) and Potassium (108 – 215 Kg /Acre) have been observed in soils of diseased plants of susceptible varieties. Comparatively higher amounts of Phosphorous and Potassium have been observed in soils of healthy plants of susceptible (P: 5.8 – 8.4, K: 103 – 500 Kg / Acre) and resistant (P: 6.3 – 8.4, K: 260 – 495 Kg / Acre) varieties. Thus, it is clear from the present study that the soils with higher amount of Nitrogen and lower amount of Phosphorous and Potassium are more conducive to fusarium wilt. While the soils with lower amount of Nitrogen and higher amount of Phosphorous and Potassium are resistant to fusarium wilt.

There is also considerable difference in the quantities of micronutrients such as Iron, Manganese and Zinc between soil samples of healthy and diseased plants (Table 1 & 2, Fig. 2). In general higher amounts of micronutrients are present in soil samples of resistant varieties. Thus higher amount of Iron (5.033to 25.19 ppm), Manganese (6.959 to 14.25ppm) and Zinc (0.289 to 0.911ppm) has been recorded in soil samples of wilt resistant and healthy banana growing areas. Soil samples of all the susceptible - diseased varieties show the lowest amount of micronutrients. Soil samples of all the healthy plants of susceptible varieties stands generally intermediate in position when the amount of micronutrients is compared.

The above results on chemical analysis of soil samples, particularly the amount of nutrients, positively correlate with the IR peaks in IR spectra (Fig. 3-5). In the most basic terms, the infrared spectrum is formed as a consequence of the absorption of electromagnetic radiation at frequencies that correlate to the vibration of specific sets of chemical bonds from within a molecule [10]. Characterization of compounds via infrared spectroscopy is not limited to organic compounds. Any inorganic compound that forms bonds of a covalent nature within a molecular ion fragment, cation or anion, will produce a characteristic absorption spectrum, with associated group frequencies. The broad bands around 3422cm^{-1} and 1641cm^{-1} are due to nitrogen compounds (i. e) N-H stretching [11], [12], [10]. The strong, broad absorption bands at 536cm^{-1} shows the presence of C-Br stretching. The strong, sharp band at 468cm^{-1} may be due to C-I stretching. The bands in the region 535-420cm^{-1} indicates the presence of halogen substituted compounds like C-Cl, C-Br, and C-I [10]. They show the presence of micronutrient elements. The peak intensities in the above regions for healthy and resistant samples differ considerably.

In the diseased samples the intensity of the nitrogen peaks seem to be more than the peak intensity of healthy samples. This may be due to the reduction of ammonia i.e amine substituted compounds. In the case of resistant samples, the peaks have much less intensity than diseased samples which proves reduction in the amount of nitrogen which results in resistance to wilt disease. The values of available N (Table 1) positively correlate with the spectral interpretations. The weak band near 2929cm^{-1} which is much weaker in healthy and resistant samples may also be due to free N-H stretching. The sharp and strong peaks around 2361cm^{-1} and 2344cm^{-1} which appear as shoulders, and the peaks in the region 1100-1000 cm^{-1} may be due to the presence of inorganic salts mainly phosphorous [10]. It may be due to P-O stretching. The major reduction of intensity in diseased sample peaks in comparison with the healthy and resistant samples confirm the major difference in Phosphorous content of the soil samples (Table 1). Increase in Phosphorus content in the soil results in increasing resistivity of banana plants to fusarium wilt.

Mineral nutrition affects soilborne diseases in many different ways. A micronutrient-deficient plant usually has depressed defense capabilities against soilborne diseases. However, in some cases, nutrients can have direct effects on soilborne pathogens.

The existence of soils that are naturally suppressive to diseases induced by soilborne plant pathogens provides good opportunities to study situations where biological control is effectively working. In most cases, suppressiveness is fundamentally based on microbial interactions between the pathogen and some populations of the saprophytic microflora. However, these biotic interactions are dependent on the abiotic characteristics of the soil. In the case of soils suppressive to fusarium wilts, it is obvious that pH and the nature of the clays are important factors interacting with the microbial populations responsible for suppressiveness. Competition for nutrients, mainly carbon and iron, has been demonstrated to be one of the mechanisms by which suppressive soils control fusarium wilts. Populations of non-pathogenic *Fusarium oxysporum* and fluorescent *Pseudomonas* spp. are, at least partly, responsible for competition for carbon and iron, respectively. Moreover, these antagonistic populations have other modes of action which can contribute to their biocontrol activity. Studies of suppressive soils suggest biological control could also be achieved by enhancing the natural level of suppressiveness that exists in every soi [13]

Disease suppression does not necessarily imply suppression of the inoculum of the pathogen. Thus, Cook and Baker (1983) [14] distinguished pathogen- suppressive soils, where the inoculum is destroyed or does not survive, from disease-suppressive soils, where inoculum is present but does not induce disease. From a theoretical point of view, suppressive soils can control disease through the effects of either their abiotic or biotic properties. Although variations of disease incidence related to soil types have been recognised for many years [2], it is only during the last 30 years that most of the mechanisms involved in disease suppression have been discovered [15].

Fig. 3. IR Spectrum of soil samples of healthy and diseased plants of susceptible varietes Mondan, Nadu & Karpuravalli

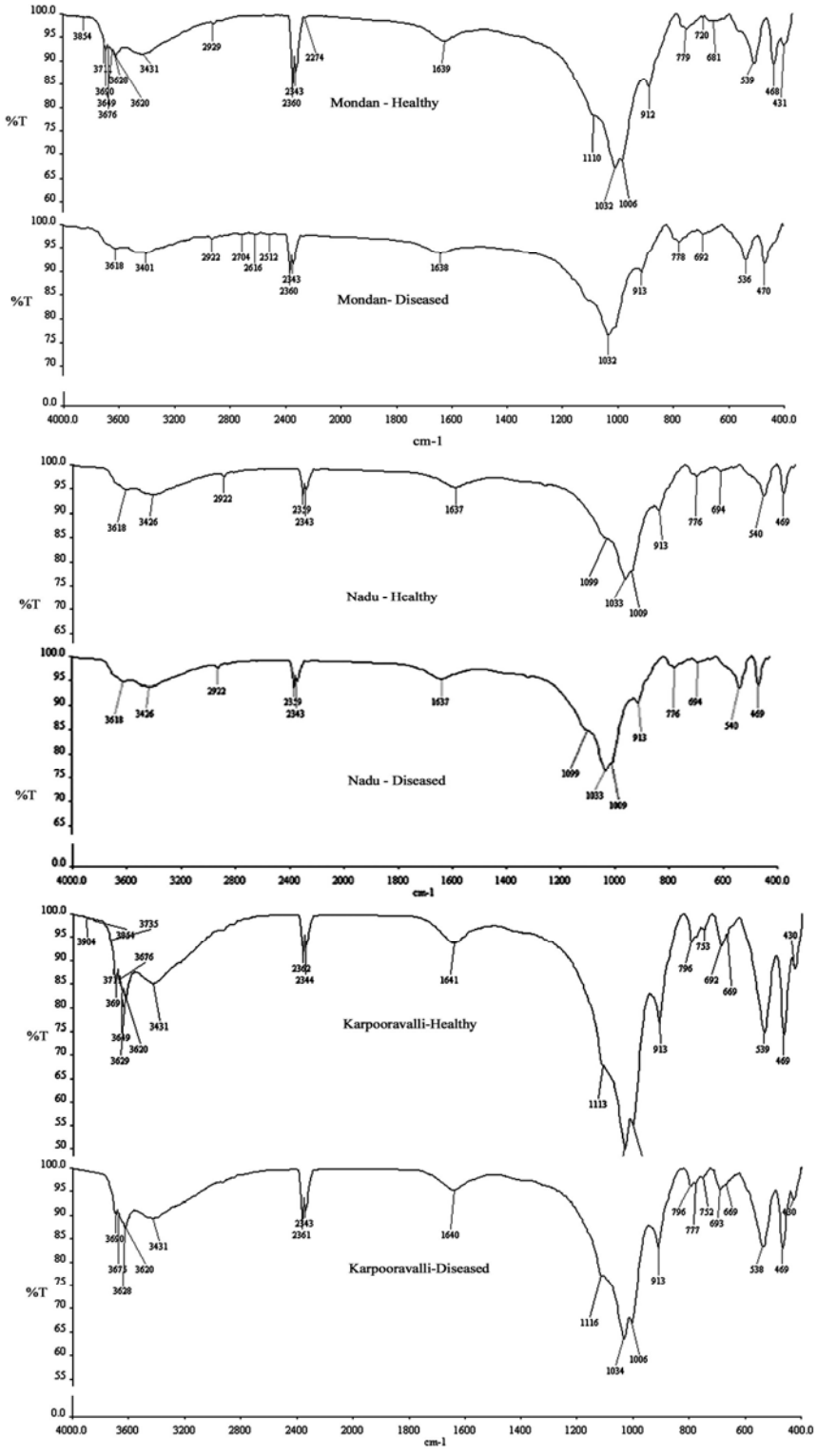


Fig. 4. IR Spectrum of soil samples of healthy and diseased plants of susceptible varietes Rasthali & Rasakathali

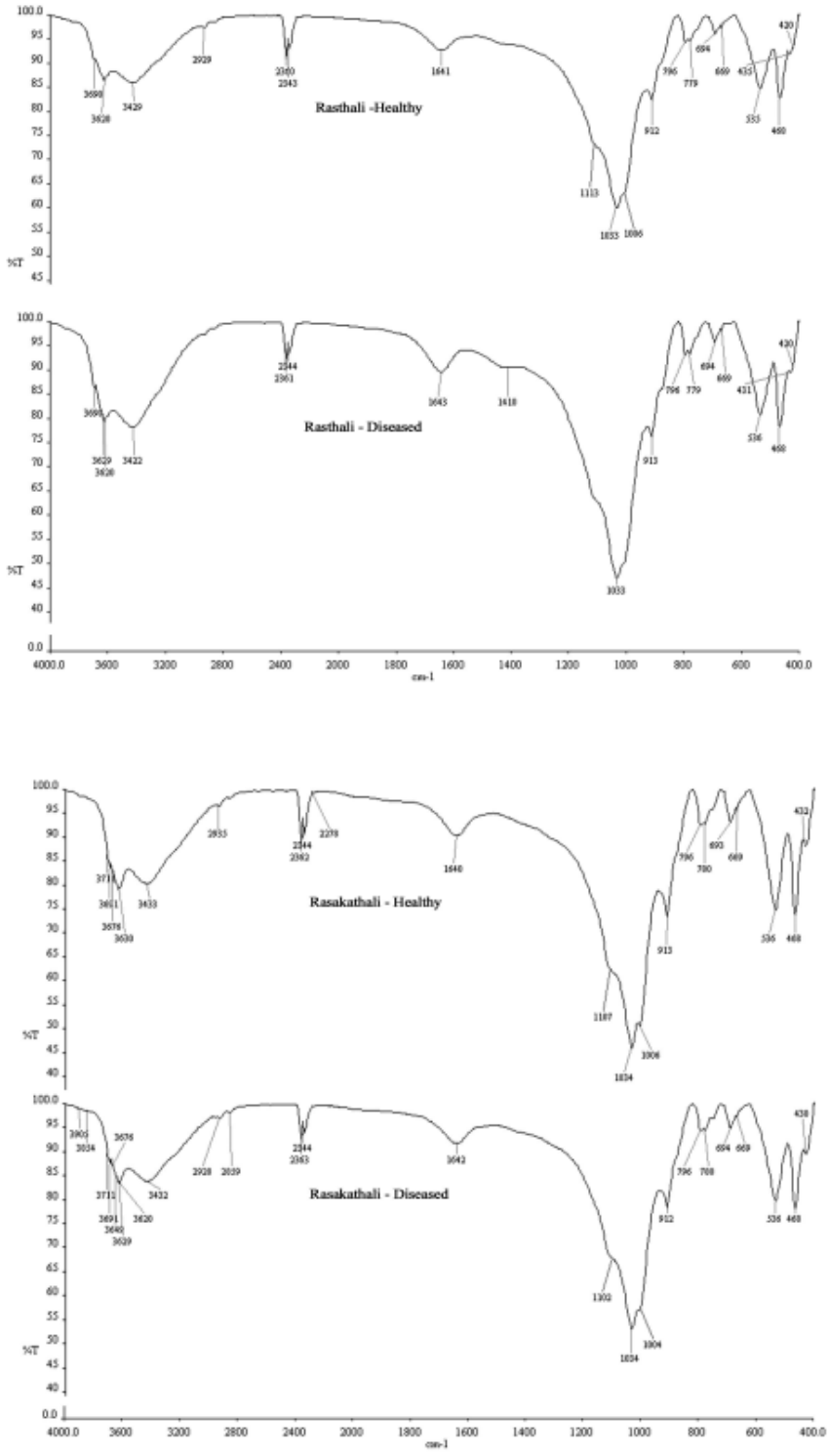
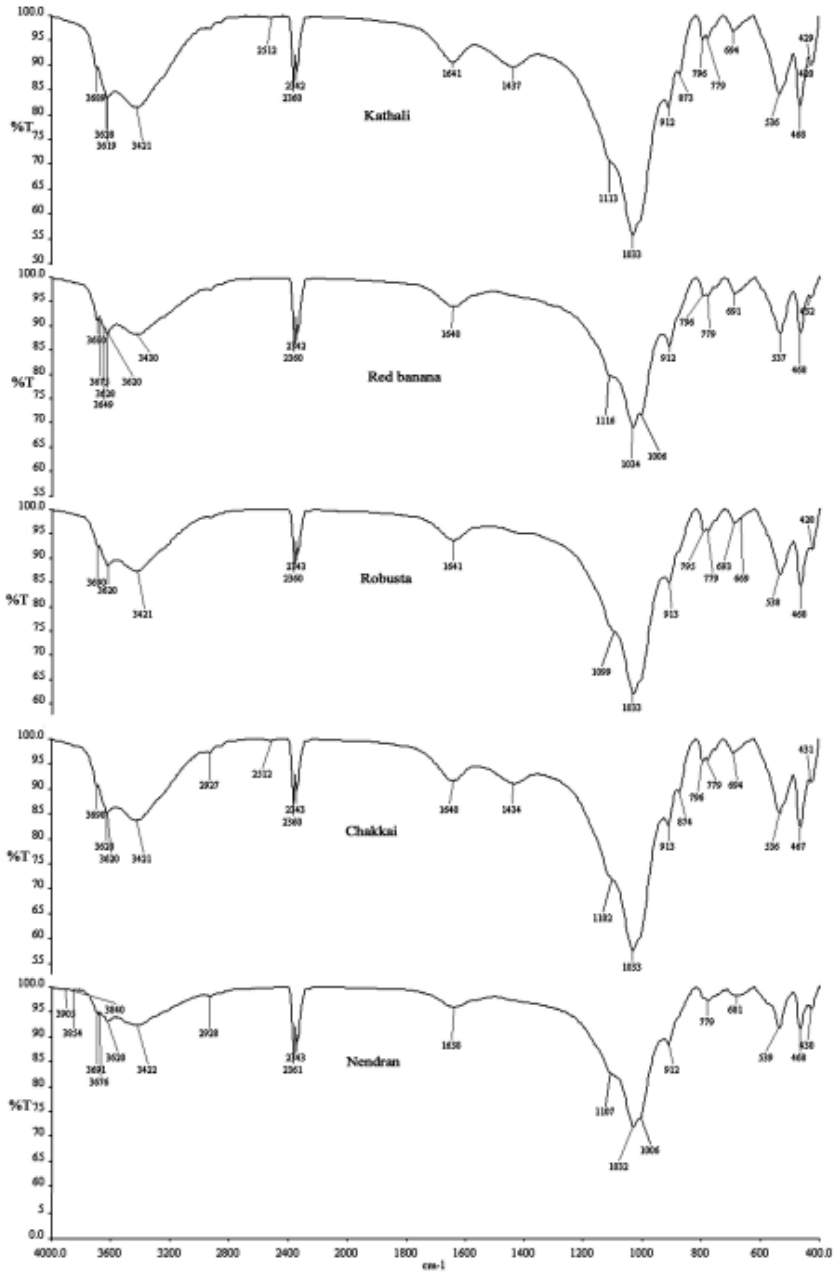


Fig. 5. IR Spectrum of soil samples of resistant varietes Kathali, Red banana, Robusta, Chakkai & Nendran



Abiotic factors such as soil texture, water potential, aeration, pH, organic matter content, cation availability (Al, Fe, Mn) are indirectly involved in the mechanisms of disease suppression, but it is difficult to generalise from one soil to another [16]. Composted organic matter generally has a potential to control soilborne plant pathogens [17]. Compost made from solid municipal waste is suppressive to several soilborne diseases and introduction of 20% of such compost in a conducive soil made it suppressive to fusarium wilt of flax [18]. There are several reports [19] to demonstrate the relationships between the disease incidence of *Fusarium* and the values of some parameters like pH, cation exchange capacity (CEC), sodium (Na) in solution, and iron (Fe). In the case of banana wilt disease, the soils are with high clay content, EC and soluble Na in suppressive areas than in conducive areas. Soil solution pH is lower in conducive areas [20]. Chlamydospore germination and Fusarium wilt of banana plantlets in suppressive and conducive soils are affected by physical and chemical factors [21]. The results of the present study on physico-chemical parameters of the soil samples also support the above concepts. The role of soil nutrients in Red Rot of sugarcane has also been demonstrated by [22]. It is found that the concentrations of Ca, Na, Fe and B are low while the concentrations of Mg, Zn, Mn and Cu are high in red rot disease resistant sugarcane growing soils. But red rot prone soils contain increased concentrations of Ca, Na, Fe and B and lower concentrations of Mg, Zn, Mn and Cu

CONCLUSION

From the present study it is concluded that the soils with higher amount of nitrogen, higher degree of electrical conductivity, lower pH (acidic), lower amount of potassium, phosphorous, iron, zinc and manganese are more susceptible to fusarium wilt of banana. The quantitative differences between soil samples of diseased and healthy plants of banana are also well expressed in IR spectra which correlate with the quantitative estimation of macro-micronutrients by classical methods.

There are several reports to prove IR spectroscopic analysis as a successful method to analyse soil samples from different habitats such as forests, agricultural fields, deserts etc. On the basis of results from soil analysis [23]. concluded that FT-IR spectroscopy is a powerful tool for the investigation of decomposition dynamics and litter quality in tropical soils. The existence of a strong nitrate absorption peak at 7194 nm (1390 cm –1) has been demonstrated by [24]. For KBr–diluted soil samples, the ratio of the area under the nitrate absorbance peak (1360–1390 cm –1) to the water absorbance peak (1640–1660 cm –1) had been proportional to nitrate concentration. [25]. have investigated the use of a relatively new signal analysis technique for the prediction of soil nitrate based on mid-infrared spectroscopy. The volume of the nitrate peak for each sample was correlated to nitrate concentration. [26]. have illustrated the characteristics of IR spectra of naturally occurring soils containing sulfates, carbonates, and nitrate. He.Y.Song *et al*. (2005) [27] have also demonstrated the Near infrared reflectance (NIR) spectroscopy as a rapid, convenient and simple nondestructive technique for quantifying several soil properties. They have used this method to estimate nitrogen (N) and organic matter (OM) content in a soil of Zhejiang Province, Hangzhou County. The present study also proves that the quick and effective method of IR-spectroscopic analysis of the soil samples of banana field will give more idea about the nutrient contents and conducive / resistant nature of the soils before cultivation of banana in a particular field.

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