Vacuum 158 (2018) 126–135

Contents lists available at ScienceDirect

Vacuum

journal homepage: www.elsevier.com/locate/vacuum

Microscopic, elemental and molecular spectroscopic investigations of root-knot nematode infested okra plant roots

Neha Sharma[a], Yugal Khajuria[a], Jitendra Sharma[a], Durgesh K. Tripathi[b], Devendra K. Chauhan[c], Virendra K. Singh[d], Vinay Kumar[e], Vivek K. Singh[a,*]

[a] School of Physics, Shri Mata Vaishno Devi University, Kakryal, Katra, 182320, Jammu and Kashmir, India
[b] Amity Institute of Organic Agriculture (AIOA), Amity University, Noida, Sector 125, Noida, Uttar Pradesh, 201313, India
[c] DD Pant Interdisciplinary Research Laboratory, Department of Botany, University of Allahabad, Allahabad, India
[d] Department of Plant Pathology, College of Agriculture, Banda University of Agriculture and Technology, Banda, UP, 210001, India
[e] Department of Physics and Astronomical Sciences, Central University of Jammu, Rahya-Suchani, Samba, 181143, J&K, India

ARTICLE INFO

Keywords:
Root-knot nematodes
Protein
Starch
Heavy and toxic metals
WDXRF spectrometry
FTIR spectroscopy
UV–Vis spectroscopy

ABSTRACT

Okra (*Abelmoschus esculentus* (L.) Moenche) is one of the warm season crops that is grown in the tropical and subtropical regions of the world. Root-knot nematodes, *Meloidogyne* spp. is major constraints in growing okra crops and reduce the crop yield. The present study was aimed to perform microscopic, elemental and molecular spectroscopic investigations of root-knot nematode infested okra plant roots. The healthy, root-knot infested and root-knot infested with fungus okra roots have been studied to study the changes in chemical composition caused by infection with root-knot nematodes. We have utilized binocular spectroscopy for microscopic observations of nematode species in infected okra plant roots. Wavelength dispersive X-ray fluorescence spectrometry has been used to detect and quantify the minerals and heavy metals in healthy, nematode infested and nematode infested with fungus okra roots. Numerous major, minor and heavy elements were quantified which have shown their toxic effects in for developing root-knot nematodes in okra plant roots. We have observed the changes in the concentrations of major and heavy elements in nematode infected okra root samples as compared to healthy root samples and in particular higher contents of Ca, K, S, Ti, Sr, and Ni is detected in nematode infected okra roots than that from healthy root samples. Changes in contents of heavy metals Al and Cr were also observed in infected roots. No significant changes have been observed in the concentrations of heavy elements Br, Ni, Rb, and Zr in healthy as well as nematode infested roots. Ag and Pd have only been detected in nematode infested okra roots. Fourier transforms infrared and ultraviolet–visible spectroscopy is employed to investigate the molecular changes in the nematode infested roots as compared to healthy roots. Fourier transform infra-red spectral studies revealed the decreasing content of starch and increased level of protein contents in root-knot infested roots as compared with healthy roots. Intensity of absorption peaks appeared in diffused reflectance spectra was observed lower for infected roots and higher in healthy roots. The present study is important for further treatment strategies of okra crop to prevent the crop loss in effective manner. The authors have utilized different techniques like wavelength dispersive X-ray fluorescence; fourier transforms infrared and ultra-violet–visible spectroscopy along with optical microscopy in plant science to study healthy and infected plant samples.

1. Introduction

Root-knot nematodes have been observed as one of the threats for global food production [1]. Due to the infestation in the tropical agriculture, the crop loss was estimated to be 5–43% [2] which depends upon the nematode species, crops and geographical regions [3,4]. In India, the annual estimated crop losses have been figured out to be nearly Rs. 242.1 billion due to major plant parasitic nematodes [5]. Most of the crop plants that accounts for majority of world's food supply and weeds are greatly influenced by root knot nematodes. However [6], reported that *M. incognita* and *M. javanica* have the widest host range infecting more than 232 and 114 genera of plants respectively. In particular, aromatic plants, pulses, fibre crops, fruits, vegetables, ornamentals, medicinal other important crops have been found affected

* Corresponding author.
E-mail addresses: vivekksingh2005@gmail.com, vivek.singh@smvdu.ac.in (V.K. Singh).

https://doi.org/10.1016/j.vacuum.2018.09.039
Received 11 July 2018; Received in revised form 5 September 2018; Accepted 19 September 2018
Available online 20 September 2018
0042-207X/

greatly by root knot nematodes. Therefore, the information on yield loss in various crops is lacking and often qualitative. 70% loss has been reported in chilli, brinjal, tomato and okra by Ref. [7] [8] reported that 82.5% plants of tomato, brinjal and okra were infected with *M. incognita* and *M. javanica* [9]. outlined losses in yields of okra (90.9%), tomato (46.2%) and brinjal (27.3%) due to the infestation with *M. incognita.*

Okra (*Abelmoschus esculentus* (L.) Moench) is one of the most economically important agricultural crops cultivated throughout the tropical and warm temperate regions of the world [10]. Commercially, it is grown in several countries including India. It has been found very rich in protein, moisture, ash, lipids, carbohydrates, fat [11] [12]; and in minerals as well such as calcium (Ca), iron (Fe), magnesium (Mg), manganese (Mn), nickel (Ni), potassium (K), sodium (Na), and zinc (Zn), [13]. Due to the presence of high quality of edible oil and high level of proteins, it is consumed as one of the protein sources [14].

Okra has also been found to be highly prone to root-knot nematodes and then the root-knot infested plants are stunted and exhibits symptoms of nutritional deficiencies, characteristics huge swellings on primary as well as secondary root systems [15,16]. Various *Meloidogyne* species attack maximum crops and greatly affects not only yields but also its quality [3]. Root-knot nematodes, *Meloidogyne* spp. are pondered as one of the most crucial pest problems affecting broad varieties of important agricultural crops globally. This disease is the prime constraints in growing okra successfully throughout the tropical and sub tropical regions. The yield loss, however, may be as high as 60% in continuously cultivated crop of vegetables due to root-knot nematode [17]. The role of root-knot nematode and their management strategies in okra crop has not been investigated particularly in Jammu region. Hence, a study was undertaken to assess the damage caused by *M. incognita* on okra.

Due to increase in continuous cultivation, nowadays all species of plants are subjected to diseases from several kinds of pathogens (like nematodes, fungi, and bacteria etc.), environmental conditions, and deficiency of minerals. Okra has been considered to be highly prone to gall nematodes and plants that are associated with this type of nematode have large galls or swelling on every part of the roots. These nematodes inject their hormones into the plant's roots and damage it by reducing its capability to absorb water and mineral nutrients and hence, interfering with photosynthates and the translocation of minerals inside the plants [18].

The sign and symptoms of root-knot nematodes are relatively undistinguished, including deficiency of mineral nutrients, stunting, incipient wilt, poor yield and most of the time plant death. Some diagnostic symptoms of root-knot affected plants exists except root gall formation, cysts, seed galls which cannot be sensed earlier and this leads to huge damage to crops which often goes unnoticed. Damage due to nematodes starts from a small area and spreads radially from the initial infection site to a large farm area. Even a lot of work has been done on the control measurements against the root-knot nematode on different vegetable crops but still the suitable measure to control nematode could not witnessed yet. Many farmers are using pesticides in order to control these diseases which are not effective and useful as their mode of action is not specific to one species. Thus, the only way to accurately diagnose nematode diseases is to study soil and plant samples from some suspected sites.

In past few years, the applications of elemental and molecular spectroscopic techniques have focused mainly on the studies of plant samples in order to get their elemental information and structural changes due to infections caused by several diseases. The species identification process involves various biological, biochemicals, morphological, molecular features, to identify the complex compounds present in the infected plants species. Advances in spectroscopic techniques such as fourier-transform infra-red (FTIR), ultra-violet visible (UV–Vis), and wavelength dispersive X-ray fluorescence (WD-XRF) spectroscopy have tackled many problems quickly associated with plants and offers the chemical and structural information and changes due to diseased infections without any specific sample preparation method. Spectroscopic technique such as FTIR has been applied to get the structural and molecular information of okra species [19]. Similarly, molecular technique such as UV–Vis spectroscopy has also been employed to identify the polyphenolic complex compounds from okra seeds [20]. Recently, Singh et al. [21], have successfully quantified the heavy and toxic elements in wheat seed gall nematodes and also differentiated the infected wheat grains affected by *Anguina trirtci* with the healthy wheat grains on the basis of the spectral changes observed employing WDXRF, FTIR, and diffuse reflectance spectroscopy. Spectroscopic studies on nematode affected okra plants have not been extensively studied to investigate the heavy and toxic metal accumulation and changes in the molecular contents. The role of root-knot nematode and their management strategies in okra crop is very much crucial. So, the present spectroscopic investigations on nematode infected okra plants were carried out at various localities of Jammu division.

Therefore, our aim was to analyze the nematode infected okra plant using spectroscopic techniques FTIR, UV–Vis, WD-XRF, and LIBS and also to compare the data with uninfected Okra plants to investigate the changes for its therapeutic strategies. Elemental profile of the okra plant samples has been achieved using LIBS and WD-XRF in order to quantify the mineral contents in okra and also to observe the changes due to the formation of galls. UV–Vis data of uninfected and infected okra plant samples was useful to investigate the spectral differences among the healthy and diseased plants. FTIR spectroscopy has been employed to investigate the chemical composition of the root-knot infested and healthy okra plant sample. The present study was very much important as literature is silent with regards of the spectroscopic investigations of the nematode infected okra plants.

2. Material and methods

2.1. Sample collection

Okra plant roots (uninfected, infected by root-knot nematodes, and infected by root-knot nematodes associated with *Rhizoctonia solani* fungus) have been collected from the highly suspected field site in Jammu from Jammu region. The infected plants were procured from the farmers fields. The collected plant samples first washed to remove the extra dirt and soil and then dried. The visual study was done to investigate the presence of root galls which can be seen from Fig. 1. Fig. 1a shows the snapshot of healthy/uninfected okra plant roots. From, Fig. 1b, one can see the clear symptoms of gall formation in roots which shows that the okra plant was infected by root-knot nematodes. Fig. 1c shows the snapshot of the root-knot nematode associated with *Rhizoctonia solani* fungus which was further identified experimentally. After drying, plant root samples were grounded using mortar and pestle in the form of fine powder. Before proceeding further characterization, the infected okra plant samples were observed and examined carefully to show root-gall nematode then further characterized using advanced spectroscopic techniques.

2.2. Characterization techniques

As accurate identification of nematodes to specific species is very crucial for their control and is a prerequisite to meaningful research. Many nematodes species are easily identified due to the appearing of large round shape galls or swells on the roots or other parts of the plant and some are difficult to identify because of their poor taxonomical description. Therefore, optical microscopy has also been used for the detection of the presence of the nematodes in the okra plant.

2.3. Characterization

The grounded okra plant samples in fine powder were characterized