ISOLATION AND IDENTIFICATION OF NEMATODE SPECIES FOUND IN FIELDS OF ONION LEAF TWISTER DISEASES IN THE BATTICALOA DISTRICT

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ABSTRACT

Leaf Twister Disease (LTD) ('Kolli kall' in Tamil) appears to be a complex problem and a major limitation in the cultivation of onion in the Batticaloa district. It was observed that LTD symptoms usually appear under rainy weather condition and up to 100% yield losses were reported in the past. Therefore, this study was conducted to identify the primary cause of LTD. Six infested fields in three major onion growing villages namely Kaluthavalai, Chettipalayam and Thettatheevu were selected for this study. Infested soil samples with damaged onion plant materials were collected at four different locations randomly in each field and examined precisely. Two hundred mounted slides of nematodes were prepared and examined to identify the nematodes based on their morphological characteristics. A number of different keys, reference collections, taxonomic catalogues and many descriptions were used in the identification.

A nematode species *Aphelenchus avenae* was identified from the soil samples and damaged onion plant materials. *Aphelenchus avenae* is a cosmopolitan ubiquitous and fungivorous nematode common in soil and decaying plant tissues.

Key words: *Aphelenchus*, onion, nematodes

INTRODUCTION

The major onion growing areas in the Batticaloa district include the villages *Viz*. Kaluthavalai, Chettipalayam and Thettatheevu. In recent past due to the wide spread of Leaf Twister Disease (LTD), it has become a serious problem concerned with profitable onion production in the Batticaloa district. Earlier it was reported from Trincomalee area in Sri Lanka that this damage was caused by onion bulb and leaf nematode (*Ditylenchus dipsaci)* (Lamberti *et al*., 1980). Later it was reported that pathogenic onion nematodes were not found in the leaves, bulbs and

soil (Ekanayake, 1988). However the LTD of onion has been reported from different parts of the world. This disease was first reported in 1924 in the Eastern part of the Pureto Rico (Chawda and Rajasb, 1996). In SriLanka LTD has been first observed in early 1980's in shallot cultivation in Kolona area and since then, this disease has gradually spreaded and caused by *Fusarium oxysporum and Colletotrichum gloeosporioides* (Rajapakse *et al*., 2001).

Recently severe attack in onion with the LTD was found in major onion growing area in the Batticaloa district. It was noted that symptoms of LTD initiated under rainy weather conditions. Symptoms were observed in most of the farmers' fields with different severity levels. The main symptoms observed in the affected plants were poor plant growth with distortions, twisting of stems and discoloration of leaf terminal portions and finally bulb rot (Figs.1and 2). It was observed that symptoms of the affected plants resembled the LTD of onion in some countries in the world (Ghawda and Rasab, 1992; Pedro Boff, 1996; Sinha and singh, 1994; Suhardi, 1993). However any records on nematodes in the Batticaloa district are not available and the identification of the nematodes associated with infected onion with the symptoms of LTD has not been performed.

Figure 1: Affected field

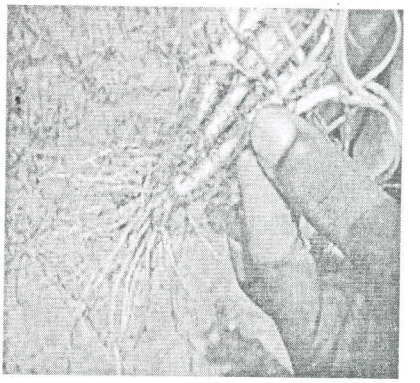

Figure 2: LTD in onion plant

In addition with the view of the serious nature of LTD and the farmers' inability to cultivate onion, this investigation has been conducted to find out the basic cause of the disease and identify the causal agents, primarily focusing on the nematodes in the disease environment (diseased onion plants and the soil)

MATERIALS AND METHODS

Isolation of nematodes

LTD affected onion plants and soil were collected from farmer's fields in Kalluthavalai, Chettipalayam and Thettatheevu areas (Fig.1). Four samples were collected from each field randomly and composited. Nematodes were isolated from bulbs and leaves of LTD affected plant by maceration technique and from soil by modified sieve method. This was performed for each field separately.

Maceration technique

The affected plant materials including roots were collected from each infested field and then it was cut into 5cm pieces and blended by using motor and pistil. The 150µm of micro sieve was placed in the Petri dish and transferred blended plant material into the micro sieve with saturated amount of Distilled water and covered. Then it was left for six hours. Thereafter, Petri dish with water was observed under the microscope to observe the presence of nematodes as well as identify the species of nematodes.

Modified sieve method

About 250gm of each infested soil was taken into a 1500ml plastic bowl. Then 500ml of water was added into it, and mixed thoroughly with hand. This mixture was filtered by 710µm sieve and then filtrate was collected in another plastic bowl. The large debris collected on the sieve were discarded. The nematodes were present in the filtrate which was used to analyze. Filtrate was again filtered by another sieve 150µm and collected in a beaker with the help of water dropping bottle. A paper towel (tissue) was placed in the micro sieve and then the micro sieve was placed in a petridish containing 30 ml distilled water and after that the collected water contained nematodes was poured into the micro sieve. It was allowed for six to eight hours and during this period the nematodes were moved from the micro sieve to Petri dish.

Identification

The collected nematodes were placed in a watch glass with the minimal amount of water and placed in an oven at 50° C until the heat-relaxed stage was formed. After that the watch glass was filled by TAF fixative containing 7ml ca40% formaldehyde, 2ml Triethanolamine and 91ml distilled water and covered. After one week nematodes were mounted on the cavity slide with glycerin and these slides were used for identification. A number of keys (Bastian, 1865, and Mathur, 1990 and Hooper 1986) were used in the identification of nematodes.

RESULTS AND DISCUSSION

Adults and different stages (juveniles) of nematodes were isolated from affected plants and soil. These nematodes isolates were mainly found from bulbs, root and root zone of onion. There were 200 mounted slides of nematodes from each field examined for the identification. The following characteristics were recorded based on the identification catalogues and descripter (Hooper, 1986, Bastian, 1865).

Body of the nematode was generally small vermiform about 1mm long, and body tapering anteriorly, female with relatively short blunt tail (figure 3)

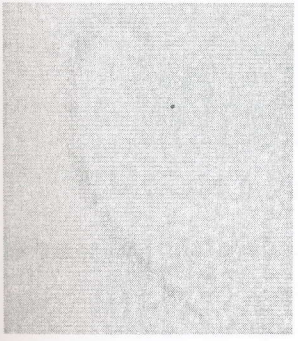

Figure 3: Body of nematode

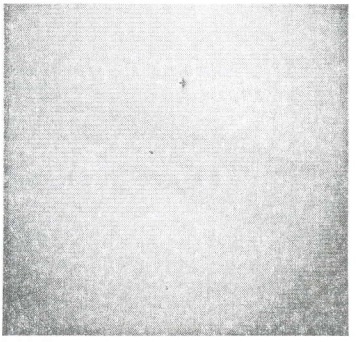

Figure 4: Head of nematode

Figure 5: Schematic diagram of Head of nematode

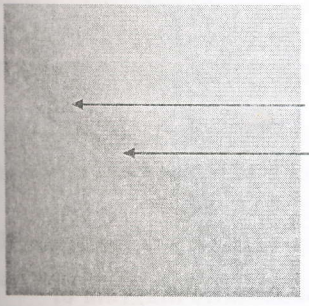

Figure 6: Median bulb of nematode

Figure 7: Schematic diagram of Median bulb of nematode

Head had no papillae, slightly offset and slightly thickening at the base. Lip region low rounded to flattened arteriorly. (Figure 4)

Osophagus consisted of a narrow procorpus, a prominent oval **median bulb** which often occupied most of the body diameter (Figure 6).

Oesophageal glands that overlaped the intestine mainly dorsally and laterally (Figure 8).

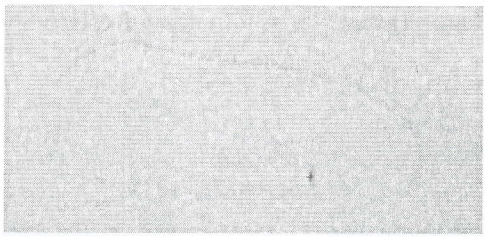

Figure 8: Oesophageal glands of Nematode

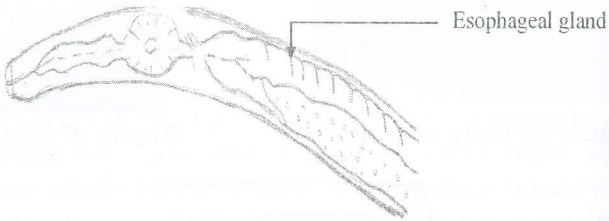

Figure 9: Schematic diagram of Osophageal glands of Nematode

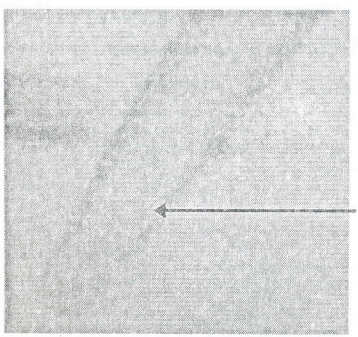

Fig.10: Stylet of Nematode

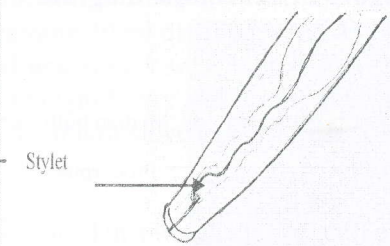

Fig.11: Schematic diagram of Stylet

Normally male with elongated **spicules**(Figure 12) and distinct 'V'shaped gubernaculum with prominent **bursa**(Figure 13) that enveloped and was supported by bursal rib which was occupied just below the tail.

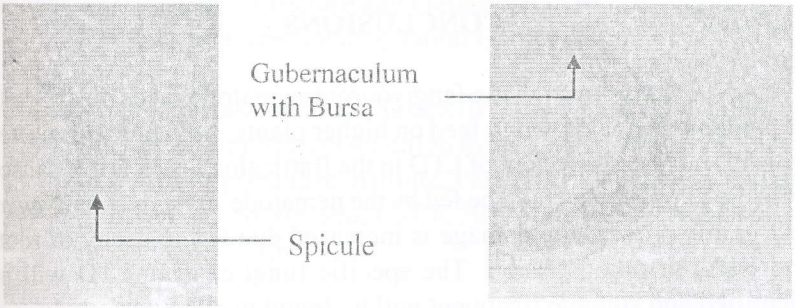

Figure 12: Spicule of nematode **Figure13: V Shaped gubernacu -lum with bursa**

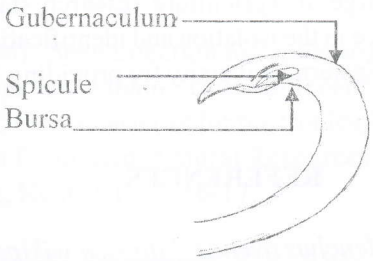

Figure 14: Schematic diagram of Gubernaculums, Spicule and Bursa of nematode

Based on the observed characteristics the nematode has been identified as the fungivorous nematode species *Aphelenchus avenae* which was associated with infested onion in the field of Batticaloa district. It is normally attracted by various fungi probably causing LTD in onion plant in the field. *Aphelenchus avenae* prefers to feed on the mycelium of the fungi, penetrates into the plant materials through the pores already created by fungi and feeds the mycelium and during this process the plant cells decay causing damage to onion plants and yield loss occurs.

The farmers use to apply carbofuran to control the nematodes and not applying any fungicides to control the fungi causing LTD.

It is assumed that LTD is caused by some fungi and the decay of roots and death of onion may be due to the damage caused by both fungi and nematode. It seems that it is a complex disease problem. It may be presumed that if the fungal attack is arrested the damage caused by nematode may be prevented as the nematode is a fungal feeder (Giannaki and Sanders, 1989, Mankau and Mankau, 1963), and this nematode is not known to feed on higher plants (Hesling, 1977).

CONCLUSIONS

The *Aphylenchus avenea* is a fangivourous nematode feeds on a variety of fungi but is not known to feed on higher plants. *Aphylenchus avenea* which is not a causal agent of LTD in the Batticaloa district, it is caused by some fungi which could be fed by the nematode *Aphylenchus avenea,* and in this process the damage is increased due to the decay of roots and bulbs of onion plants. The specific fungi causing LTD will be identified and suitable treatment will be found in our future study.

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REFERENCES

Bastian. (1865). *Aphelenchus avenae*. http://en.wikipedia.org/wiki/Aphelenchus *avanae* (Accessed on 20th Feb 2008).

Chawda,H.T. and Rajasab,A.H. (1996). Onion Anthrachnose Disease Symptoms: a review:*Onion newsletter for the tropics*, Eds.Lesley Currah and John E orchard, Natural resources Institute, Chatham, Maritime, Kent, UK. 7: 82-84.

Ekanayake,H.M.R.K. (1988). Red onion disorder-reported from Ratnapura Distric. Division of Entomology, Horticulture Research and Development Institute, Gannoruwa, Peradeniya, Sri Lanka.

Giannakis, N. and Sanders, F.E.(1989).Interactions Between Mycophagous Nematodes,Mycorrhizal and Other Soil Fungi. Agric.Ecosyst.Environ.29, 169-167.

Hesling,J.J. (1977). *Aphelenchoides composticola.* In C.I.D. Description of Plant-Parasitic Nematosed. Eds.S. Willmott, P.S. Gooch, M.R. Franklin. Set 7, No. 92. Commonwelth Agricultural Bureaux, Farnham Royal, Slough, U.K.

Hooper, D.J., (1986). Structure and classification of Nematodes, Rothamsted Experimental station Harpenden

Lamberti F., Ekanayake H.M.K., Sasanelli N. and Larizza A. (1996). *Apelenchoides besseyi* on onion in Sri Lanka, Research Report of Central Agriculture Institute, Peradeniya, Sri Lanka, *Istituto di Nematologia Agraria, C.N.R:70126 Bari, Italy.* Nematal medit.(1996),24;63-71 .

Mankau,R. and Mankau,S.K. (1963). The role of mycophagous nematodes in the soil. I. The relationships of *Aphelenchoides avenae* to phytopathogenic soil funi. *In* Soil Organisms. Eds.J.Doeksen and J van der Drift. Pp 271 – 280. North Holland, Amsterdam, The Netherlands.

Pedro Boff. (1996). Seed infection by *Colletotrichum gloeosporioides* F.Sp. Cepa is the main cause of recent onion bulb rot South Brazil. Onion newsletter for the tropics, Editors: Lesley Currah and John E Orchard, Natural Resources Institute, Chatham, Martime, Kent, UK, 7: 16-17.

Rajapakse, R. G. A. S., Edirimanna, S. P., Weerarathna, G. and Yappa, D.R. (2001). Management of leaf twister disease of Kolonna area (IM_2 agro-ecological region). *Annals of the Sri Lanka Department of Agriculture* 3: 201-210.

Sinha, J.N and A.P. Singh. (1994). Seven curl or twister disease of onion in India, Indian Phytopathology. 47:214.