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Pallabi Roy
Department of Nematology, Assam Agricultural University, Jorhat, Assam, India

Abhijit Chetia
Department of Nematology, Assam Agricultural University, Jorhat, Assam, India

Madhumita Goswami
Department of Nematology, Assam Agricultural University, Jorhat, Assam, India

Corresponding Author:
Pallabi Roy
Department of Nematology, Assam Agricultural University, Jorhat, Assam, India

Histopathological study of root-knot nematode, *Meloidogyne incognita* infested roots of ash gourd, *Benincasa hispida*

Pallabi Roy, Abhijit Chetia and Madhumita Goswami

Abstract
Ash gourd, *Benincasa hispida* is a cucurbitaceous crop. Ash gourd is also known as Safed Kaddu, white pumpkin, winter melon etc. It is extensively cultivated as sole crop or as a mixed border crop almost all the year round throughout the India. It is fairly rich in minerals and contains little of vitamin B and C. Ash gourd is known for its medicinal values. Because of its low calorie nature, it is ideal for diabetic patients and the people seeking weight control. Root-knot nematode, *Meloidogyne incognita* is one of the destructive pests of ash gourd. It disrupts the physiology of the plant and arrests the growth of the plant and reduces yield and quality. Histopathological study of the infected roots revealed formation of giant cells, eggs and presence of second stage juveniles inside the roots.

Keywords: Ash gourd, giant cells, histopathology, infestation, root-knot nematode

Introduction
Ash gourd, *Benincasa hispida* is a cucurbitaceous crop. It is believed to be originated in Java, Indonesia. Ash gourd is also known as Safed Kaddu, white pumpkin, winter melon etc. It is a perennial crop. Leaves are simple, alternate, large, with numerous hairs; flowers yellow large and unisexual; fruits cylindrical, hairy, and covered with ashy powder throughout. Fruits contain numerous white coloured embedded seeds. Fruits are large, succulent, and densely hairy when young, with a thick waxy deposit when mature. It is a popular vegetable crop, especially among Asian communities both for nutritional and medicinal purposes. It is preferred as a cooked vegetable, boiled alone, boiled with meat, or included in a variety of dishes. In India, it is also used in making sweets called peda and burfi. Ash gourd plant is used in medicines to treat cardiac diseases, respiratory diseases, gastrointestinal problems and urinary diseases. Fruits are traditionally used as a laxative, diuretic, blood disease, jaundice, epilepsy, schizophrenia and other psychological disorders. The major constituents of fruits are volatile oils, flavonoids, glycosides, saccharides, proteins, carotenes, vitamins, minerals, ß-sitosterin and Uronic acid.
Root-knot nematode, *Meloidogyne* spp. is a serious pest of ash gourd. Symptoms include-yellowing of leaves, stunted and patchy growth in the field. Gall formation and presence of egg masses are the diagnostic symptoms of root-knot nematode infestation. Heavy infestation cause rotting of the roots, reduction in yield and quality of the fruit. Therefore, studies were conducted to observe the histopathological changes due to the nematode infestation.

Materials and Methods
Root-knot nematode infested plant samples were collected from the cultivated field of Jorhat. For staining of nematodes in plant tissues, acid fuchsin in lactophneol method was used. (Byed *et al.*, 1983) [2]. The stained galled roots were teased under stereoscopic binocular microscope and adult females were collected in a glass slide. The standard procedures have been followed to cut and mount the perineal pattern of the root-knot nematode and observed under microscope.
The roots were thoroughly washed under running water to remove soil and sand particles adhering to roots. In order to remove sand particles attached by matrices around the female nematodes, 35-45% hydrogen peroxide was used for 1-2 minutes. The roots were cut into bits of 0.5-1 cm and fixed in F.A.A (Formalin- 6.5 ml, acetic acid glacial- 2.4 ml, ethyl alcohol 50%- 100 ml). Then allowed the roots to fix into this solution for 24 hours. The root bits were submerged in a solution containing 5ml of acid fuchsin stain and distilled water to 100ml

F.A.A solution and submerged the F.A.A. fixed roots for 20 minutes. Then washed in 50% alcohol. The fixed root bits were washed under running water for 30 min.

For dehydration, the roots were passed through 50, 70, 80, 90, 96 and 100% ethyl alcohol for minimum 30 min. or 1 hour each. The roots were transferred through xylene and alcohol mixture series 1:1, 3:1 and 1:0 for 30 min each. The roots were kept in the mixture of xylene and paraffin wax in oven at 60°C (at melting point of paraffin wax) as per following sequence: xylene + paraffin wax (1:1); xylene + paraffin wax (1:3) for overnight each with lid container open. Change the root bit 3 times in pure paraffin wax at an interval of 24 hours so that embedding material enters the cells of roots.

Prepared a block by placing root material in the centre of block and mould filled with molten paraffin wax. Carefully the root bits were placed exactly in the center so that paraffin wax remains all around the root. Trimmed the blocks to remove the unwanted paraffin wax around the roots and orientation of the root was seen within the block. The block was fixed in block holder. The block was properly oriented. The angle of block holder was fixed on the microtome to get precise transverse or longitudinal sections of roots. Calibrated the microtome for getting sections of desired thickness. Normally 3-5µm thick sections provides very fine cellular details and 10-15 µm tick sections are useful for general anatomical details. Fixed 3-4 ribbons containing root sections on a glass slide previously smeared with Meyer's albumen freshly prepared with white of egg + glycerine (1:1). Meyer's albumen was preserved by adding small quantity of sodium salicyclate as preservative. Stretched the ribbons after adding few drops of water on slide and put them on hot plate at 35-40°C. Dried the slide by keeping them upside down (ribbon facing downward) preferably overnight at room temperature.

Removed the wax by putting dry slides with stretched ribbons in xylene. Changed two times for 15 min. each. Passed the slides through 100, 96, 90, 80, 70, 50, 30% alcohol and water (5min. each) for hydrating the sections. Stained the sections in 1% aqueous safranin for 24-48 hours. Stock solution of safranin was prepared by dissolving 1g stain in 100 ml ethyl alcohol (96%). Stock solution was diluted with equal amount of distilled water. Removed the excess of stain by rinsing sections in water for 5 min. Dehydrated the sections by giving successive bath in 30, 50, 70, 80, 90 and 96% alcohol for 5 min. each. Countered stain the sections in 0.1% Fast Green stain FCF in 96% alcohol. Passed the sections in 96% absolute alcohol, absolute alcohol and xylene mixture (1:1 ratio) for 3 min. each. Changed the section two times in xylene for 35 minutes each and mounted in DPX mountant. Placed the slides in an oven at ± 45°C overnight. In this way the slide becomes ready for observation

Results and Discussion

The infested roots of ash gourd showed typical symptoms of root-knot nematode infestation. Heavy galling and egg mass were observed on the roots. Leaves showed chlorosis symptom. The study of perineal pattern revealed the nematode as *Meloidogyne incognita*. Histopathological studies showed the second stage juvenile of *M. incognita* inside the cortex, second stage juvenile is the infective stage. This stage penetrates the roots with the help of stylet and enters into the roots. Giant cells were observed around the head of the adult female nematode, these giant cells are the feeding site of the nematode. The shape of the giant cells were not uniform, some were ovoid and some were irregular in shape. Deformed vascular tissues were observed. Giant cells are multinucleated with large vacuoles which act as metabolic sink supplying nutrients for the developing female and throughout its parasitism. Similar observation was recorded by Dropkin (1969) [4] in tomato. The eggs and hatched juveniles were found in the necrotic rings and second stage juveniles were found moving out of the necrotic rings in search of healthy tissues. Similar observations were also recorded by Dipali *et al.* (2014) [3].

Fig 1: Root-knot nematode, *Meloidogyne incognita* infested ash gourd plant

Fig 2: Perineal pattern of *M. incognita*

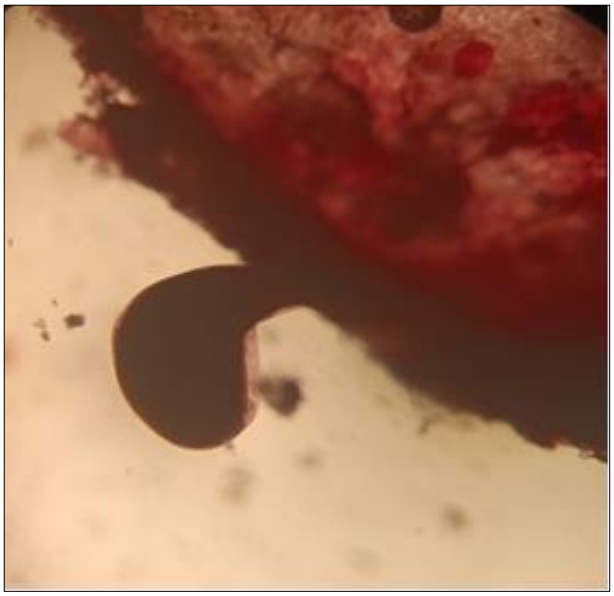

Fig 3: Adult female nematode

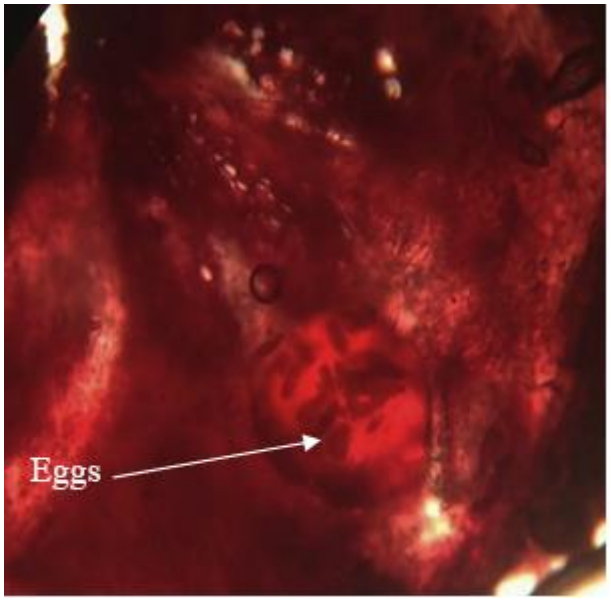

Fig 4: Presence of eggs inside the roots

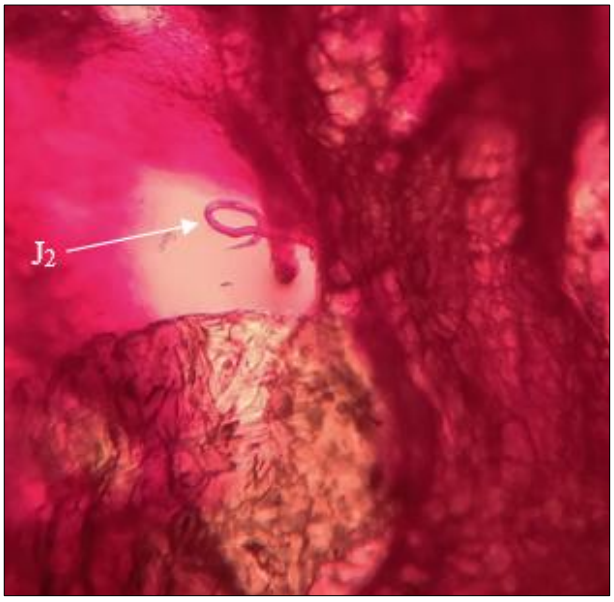

Fig 5: Second stage juvenile (J_2)

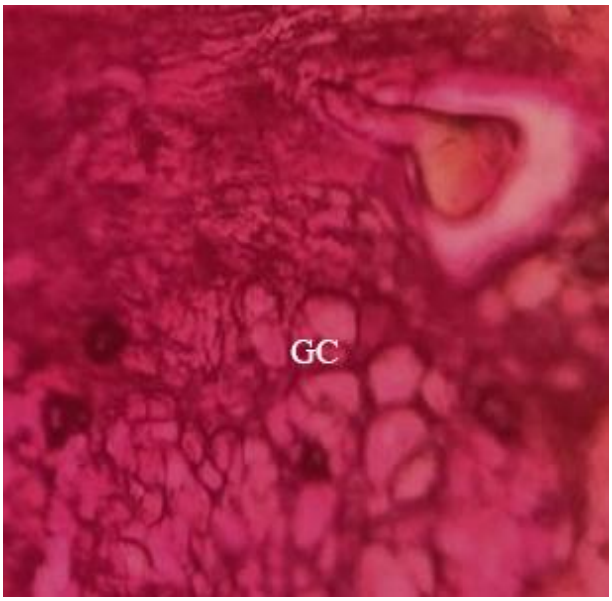

Fig 6: Formation of giant cells (GC)

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