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Root-knot nematode - a threat to brahmi (*Bacopa monniera)*

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ABSTRACT

Generally leaves, roots, flowers, barks of brahmi are most commonly used raw materials and interestingly these parts of the plants are more susceptible to the attack of insect-pest, diseases and nematodes and as a result quality of the raw materials which ultimately lead to the economic losses. During a survey programme this brahmi plant was found to be susceptible for root-knot nematode. The infected plants show the characteristics symptoms of stunted growth in patches, yellowing and drying of the plants from the tip and when infected plants uprooted small galls were observed in the roots. The small knot like galls were appeared in the main as well as on the lateral roots, the size of the galls was about 0.25-0.5 cm in size. Histopathology of the infected roots revealed that the second stage juvenile (J_2) initially penetrated in the root cortex and then move to the cortical layer of the cells and started feeding. During the feeding process they developed metabolically highly active permanent feeding cells in the vascular system of the plant by the hyperplasia and hypertrophy of the parenchymata cells and thus thereby affecting the translocation of the nutrient to different parts of the plants and leading to the collapse of the plants.

Keywords: Brahmi, root-knot nematode, histopathology, feeding cell,

INTRODUCTION

North-east India which is ecologically represented by the Eastern Himalayan biome and one of the biodiversity hotspot which harbours numbers of endemic and extinct flora and fauna (Schedule I species) as compared to other parts of India. According to an estimate, medicinal and aromatic plants occupy an area of about 2,50,000 hectares producing raw materials worth Rs. 500 crores, generating business opportunities of more than Rs. 5000 cores. According to WHO the global market of medicinal and aromatic plants as herbal product is estimated to be US $ 62 billion and it is expected to reach to the tune of US $ 5 trillion by 2050 (Anon, 2009). With the increasing interest in the use of natural products of plant origin for pharmaceutical industries there is also concerned on quality of the raw materials. Generally leaves, roots, flowers, barks are most commonly used raw materials and interestingly these parts of the plants are more susceptible to the attack of insect-pest, diseases and nematodes and as a result quality of the raw materials which ultimately lead to the economic losses.

Bacopa monniera commonly known as "brahmi", is an annual succulent, creeping plant with numerous branches, rooting at the nodes belonging to family Scrophulariaceae and grows naturally throughout the North-eastern states of Indian subcontinent in wet soil, shallow water, damp and marshy areas. The literal meaning of the word "Brahmi" is "energy or shakti of Brahman"**.** Brahmi was used in traditional medicine from centuries ago and presently it is used as a brain and mental tonic to treat Alzheimer disease, memory loss, insanity, insomnia and other mental illness. It has great value in Ayurvedic medicine due to the present of active constituents such as alkaloids, sterols, saponins, betulic acid, stimastorol, betasitosterol, bacoside and bacopa saponins. The active constituent bacoside has released the naturally occurring nitric oxide which relaxes the aorta and vein allowing smooth blood flow that nourishes the nerve cells, improving cognitive capability; promote mental clarity and focus and thus this plant become most important in pharmaceutical industry.

These medicinal plants are highly susceptible to number of insect pest, diseases and nematodes. A very few information are available on the nematode pest of medicinal plants particularly on Brahmi. Deuri *et.al.* (2013) reported the present of root-knot nematode from the roots of Brahmi plant from Jorhat district. Since the Brahmi is the second in the list of most traded medicinal plant, therefore an effort was made to study the morpho-anatomical changes in the roots of Brahmi due to the infection of *Meloidogyne incognita*.

MATERIALS AND METHODS

Histopathology

During a random survey programme, roots of *M. incognita* infected Brahmi plants were collected from cultivated as well as non cultivated areas of Jorhat district. The roots were cut into bits of 1-2cm length and fixed in F.A. A. (Formalin Acetic-Alcohol) and processed for histological studies according to standard procedure given by Sass (1964). Dehydration was carried out in a serial concentration of ethanol (50, 60, 70, 80, 90 and 100) per cent for 2-3 hrs in each solution. Then it was cleared in a mix of absolute ethanol and xylol (3:1) for 3 hrs and subsequently passed through 2:2 and 1:3 ethanol and xylol. The dehydrated root tissues were infiltrated with paraffin wax (60°C) to replace alcohol and then embedded in clear paraffin and made into blocks which were turned into turned after hardening. Sections of 10-15µm thickness were cut with a rotary microtome on a clean glass slide smeared with 'Mayer's albumin'. The ribbon sections were placed on 2-3 rows on clean glass slide. A few drops of water were added and the ribbon was stretched by passing the slide on the flame of a spirit lamp. The slide was dried overnight in an incubator at 40°C.

Processing of slide

The wax of the ribbon in the slide was dissolved by dipping in xylol for 2-5 minutes. The slide is then passed through absolute ethanol (95, 70, 50, 30) per cent and distilled water for 2-5 minutes in each solution. The slide was stained for 1-2 hrs in 1 percent Saffranin solution. The excess of stain was washed in distilled water until the slide was then passed through (30, 50, 95) per cent ethanol for 5 minutes in each solution. It was then counterstained with fast green for 5-30 second. Then the slide was passed through absolute ethanol and was cleared in cloved oil for 5-10 minutes and then the slide was mounted in DPX mounting. DPX mounting is a chemical used to observe a nematode finally in the microscope.

RESULTS AND DISCUSSION

The brahmi plants were found to be susceptible for root-knot nematode during the survey programme. The infected plants show the characteristics above ground symptoms of stunted growth in patches, yellowing and drying of the plants from the tip and when the infected plants were uprooted small galls were observed in the roots (Fig. A, B, C), which is the typical symptom of root-knot nematode infected plant.

The second stage juvenile is the infective stage of root-knot nematode. At this stage, juveniles of *M. incognita* initiated the penetration by puncturing action of stylet and entered into the roots (Fig. D). The transverse sections of roots exhibited severe infestation of nematode. After penetration, the 2nd stage juveniles were seen migrating towards vascular zone and then slowly settled down near the cortex (Fig. E). After settling down, the J_2 stage moults to J_3 and J_4 stages in feeding site and finally to adult females. Voracious feeding by females in adjacent cells caused cell damage.

After settling down, the J_2 stages moulted to J_3 and J_4 stages and were seen at one feeding site. These were finally developed into adult females. Females feeding in adjacent cells and caused cell damage thereby disintegrating cortical and vascular tissues.

The nematodes in vermiform stage remained oriented parallel to the vascular strands after development into mature females and then became perpendicular to the vascular strands. Development of nematode, formation of giant cells, hyperplasia and hypertrophy affected all kinds of tissues and apparently, it became difficult to locate endodermis, pericycle and structures of normal xylem and phloem. The findings are in conformity with Khan *et al*, (2010) on apple roots, Sayed *et al.* (2010) on mango roots and Robab *et al.* (2010) on soybean roots infected by *M. incognita*. The giant cell complex was induced near the nematode head but hypertrophied and hyperplasia cells were observed at a little distance surrounding the body of nematodes. The shape and size of giant cells were not uniform, some were ovoid, elongated or irregular shaped with dense cytoplasm and multinucleated condition (Fig. F). Occasionally the common cell wall between a giant cell and parenchyma cells was dissolved and this acts as

Fig. A Patchiness of the infected plants

Fig.B Chlorosis and yellowing of infected plant

Fig.C Galls form in the root of Brahmi plant

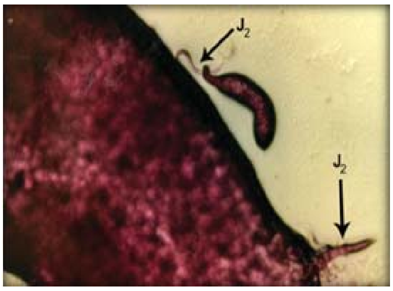

Fig. D Transverse section of brahmi roots showing partial penetration of second stage juvenile (J_2) of *M. incognita* **into the root**

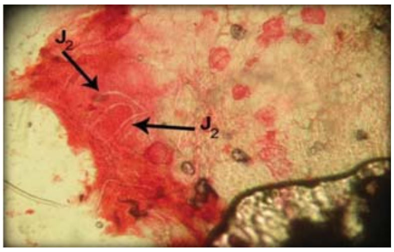

Fig. E Transverse section of brahmi roots showing partial penetration of second stage juvenile (J_2) of *M. incognita* into the root

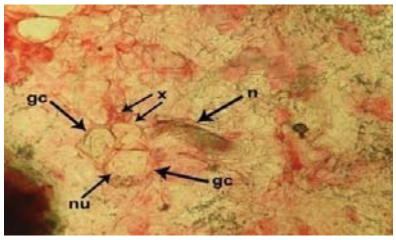

Fig. F Transverse section of brahmi roots showing *M. incognita* (n), Giant cell (gc), nucleus (nu) and broken cells (x)

feeding sites for adult female nematode. Deformation of vascular tissues at and near the feeding sites was a common observation. Hypertrophy and hyperplasia was commonly observed in both the cortical and epidermal cells. The adult females were seen laying egg masses in gelatinous matrix embedded in cells. Fully developed females with egg mass were usually surrounded by necrotic tissues causing onset of necrosis in the roots. The eggs and hatched juveniles were found within the necrotic rings and J_2 were found moving out of the necrotic ring in the starch of healthy tissue. These observations are supported by the findings of Dipali *et al.,* (2014) with similar developments in brinjal roots infested by root-knot nematodes.

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