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OCCURRENCE AND DISTRIBUTION OF ROOT –KNOT NEMATODE AND FEW FUNGAL PATHOGENS FROM PATCHOULI GROWING AREAS OF ASSAM

Nematology

KEYWORDS: Assam, *Cercospora* sp., *Fusarium oxisporum, Meloidogyne* incognita, Patchouli, *Rhizoctonia solani*

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ABSTRACT

Patchouli (*Pogostemon cablin*) is an important aromatic plant belonging to family Lamiaceae. This crop is in high demand for its essential oil. Root-knot nematode (*Meloidogyne incognita*) is reported to be the most important pest of patchouli in North East India. Along with root-knot, wilts causing fungal pathogens are also involve in causing huge loss in foliage production. Survey on occurrence and distribution of root knot nematode in patchouli from different districts of Assam reveals the presence of root-knot nematode in almost all patchouli growing areas. Maximum frequency of 38.88 per cent occurence was recorded in Nagaon district, whereas minimum frequency of 21.87 per cent was recorded from Golaghat district. Three fungal pathogens were found to be associated with root-knot infested plants *viz., Fusarium oxisporum, Rhizoctonia solani* and *Cercospora* sp. Both nematode and fungus together, were found to aggravate the disease condition of the plants.

INTRODUCTION

Plants are the richest resource of drugs of modern medicines, traditional systems of medicine, food supplements, pharmaceutical, intermediates, folk medicines, and chemical entities for synthetic drugs (Hammer *et al.,* 1999 and Bishnu *et al.,* 2011). Patchouli [*Pogostemon cablin* (Blanco)] is an important aromatic plant belonging to family Lamiaceae, is a native of humid Indo-Malayan tropics. (Arpana *et al.,* 2008). It is grown wild in Malaysia, Indonesia, Singapore and few other countries. The plant is small bushy herb which yields fragrant leaves containing very sweet smelling oil. It is used to manufacture soap, cosmetics and incense and for imparting lasting oriental perfumery notes to many fancy products. It is also used in flavoring baked foods, meat and sausages, etc. (Singh *et al.,* 2009 and Puttanna *et al.,* 2010). The shade dried leaves of patchouli on steam distillation yield an essential oil, a natural source of patchouli alcohol, valued by perfumers (Rekha *et al.,* 2009) for its fixative properties.

Indian demand for patchouli oil is around 220 tonnes valued at 34 crores while global demand is to the tune of 1600 tonnes of oil per annum with a value of 240 crores (Vijaykumar, 2004). It is grown in small pockets in Karnataka and Tamil Nadu and few places in Assam and West Bengal.

Production of patchouli oil in India is limited to only 10-15 tonnes/annum. Low area of cultivation, disease infestations are the key factor for low production. Many workers have identified root-knot nematode to be the most important pest of patchouli followed by wilt causing pathogens (Narayanappa *et al.,* 1984; and Sreedevi, 2009). Sheshadri (1970) was first to report specifically that patchouli raised over an area of 20 hectares by the Government Cinchona Department, Tamilnadu, at Annamalai was completely wiped out by *Meloidogyne*. The severity of this nematode is much more in the hot humid climate of Assam (Ahmed, 2002). In rainy and wet weather conditions, root knot infestation leads to sudden wilting of the plants and dies within 2-3 days due to secondary infection by fungal and bacterial pathogens.

Considering the need of protecting this high valued crop, and increasing productivity of patchouli in the North-East part of India, present investigation was undertaken with the following objectives.

1. Survey of root-knot nematode in patchouli, from patchouli growing areas of Golaghat, Nagaon, Jorhat and Sonitpur districts of Assam

2. Identification of fungal and bacterial pathogen associated with root-knot nematode infected patchouli

Materials and Methods

The study on the occurrence and distribution of root-knot nematode *Meilodogyne incognita* in patchouli, was carried out in four patchouli growing districts of Assam. Laboratory work relating to culturing, maintaining and identification of nematode, fungal and bacterial pathogens were carried out in the Department of Nematology and Department of Plant Pathology, Assam Agricultural University, Jorhat, Assam.

Collection of samples

A random roving survey was conducted in Golaghat, Nagaon, Jorhat and Sonitpur, district of Assam during 2014-2016. Soil and root samples showing root galls, plants showing typical symptoms of wilt and leaf spots and / leaf necrosis were collected from the patchouli growing areas. Soil and root samples were put in polythene bags, tied with a twin thread with labels. Leaf samples were put in separate polythene bags, tied and labeled.

Extraction of nematodes, killing and fixing

Extraction of nematodes from soil was done by modified Cobb's Sieving and Decanting Technique (Christie and Perry, 1951) where a series of sieves (20, 60, 150, 250 and 350) were used for extraction of nematode from soil. Killing and fixing of nematodes was done by treating nematodes with 8 per cent boiled formalin solution.

Staining of nematodes

For staining of nematodes in plant tissue, Acid fuchsin in lactophenol method was followed (Byed *et al.*, 1983).

Preperation of perennial pattern

The stained galled root tissues were teased under a binocular microscope and adult females of root -knot nematodes were collected on a clean glass slide. Standard procedures have been followed to cut and mount the perennial pattern of the root knot nematode. The perennial pattern was observed under oil emersion layer in a compound microscope.

Nematode culture

Single egg mass of *Meloidogyne incognita* was collected from the pure culture already maintained in tomato plant in the Nematode Culture House of Department of Nematology, Assam Agricultural University, Jorhat. Egg mass was inoculated to young patchouli plants grown in cemented pots with sterilize soil and were maintained in the Glass House for build up of nematode population for subsequent use.

Isolation and maintenance of pathogens associated with root knot nematode

Leaf and stem specimens were brought to laboratory of Mycology Research Section, Department of Plant The fungal disease specimen, washed with tap water and then rinsed with distilled water. Rotted roots and stem portion from the collar region of wilted plants and diseased leaves showing leaf spot symptom were cut into pieces and then surface sterilized with 0.5 per cent $NaOCl_2$ for 1 minute. These were then washed with 3 changes of sterile distilled water and dried in sterilized blotting paper. The sterilized pieces of roots and leaves were then placed on Potato Dextrose Agar (PDA) media and incubated at 28±1°C.

Fungal pathogen was isolated by hyphal tip culture method on PDA. The pathogens were identified based on its mycelial and conidial characteristics following standard mycological keys (Barnett and Hunter, 1972) and were maintained separately on PDA medium for further studies.

Result and Discussion

The random roving survey conducted in patchouli growing areas of four district of Assam (Plate 1) revealed that root -knot nematode was prevalent in almost all the surveyed areas. The roots were moderate to profusely galled (Plate 2), infected plants were pale green, stunted and exhibited unthrifty growth. Incipient wilting was observed in such plants. In many of the nematode infested plants the leaves were reddish and reduced in size (Plate 3). Microscopic examination of the galls revealed the presence of adult females, eggs and juvenile stages of *Meloidogyne sp* in the vascular region of roots. The female was pyriform in shape while males were vermiform. The presence of egg masses outside the gall was also noticed in patchouli roots.

In plants with both nematode and fungal infection, these symptoms were severe in intensity and during rains these plants wilted and died abruptly. Whereas plants with only nematode infestation lived for a little longer time with an unthrifty growth.

The study of perennial pattern revealed oval, high, irregular arch composed of closely spaced wavy lines. Interior portion of the arch above anus marked by numerous zigzag and broken striae which sometimes form a whorl. Often there are short transverse striae extending from the inner striae towards the vulva. Vulva and anus were prominent (Plate 4). These characteristics confirm the root-knot species to be *Meloidogyne incognita* (Kofoid and White, 1919). The relative frequency of occurrence of root knot nematode was observed to be 21.87, 38.88, 25.00 and 33.33 per cent in Golaghat, Nagaon, Jorhat and Sonitpur district of Assam respectively (Table 1). In Nagaon district, the patchouli cultivating area is more as compared to other districts. It is in cultivation from more than 20 years back. Maximum frequency of occurrence in Nagaon, may be due to the continuous build up of root-knot population with the crop from a long time as compared to the other districts where patchouli cultivation is relatively new.

From the surveyed samples, three fungal phytopathogens viz., *Fusarium oxysporum*, *Rhizoctonia solani* and *Cercospora* sp have been isolated. Symptoms such as wilting, leaf spots and infection in collar region were found in collected diseased samples (Plate 6, 10 and 14). Pure cultures of the pathogens maintained in Potato dextrose agar media (PDA) (Plate 5, 9 and 13), Characteristics of *Fusarium* sp. observed under compound microscope are conidiophores were short, simple or branched. Microconidia abundant, mostly 1-celled, hyaline, oval to ellipsoidal or sometimes ablong (Plate 7 a). Macroconidia hyaline, fusiform, moderately curved, pointed at both ends, mostly 3-septate but sometimes upto 5-septate (Plate 7 b), Chlamydospores terminal or intercalary (Plate 8).

Fungal pathogen isolated from leaf spot was identified as *Cercospora* sp. (Plate 9) and it was confirmed by studying the microscopic characters such as characteristics of conidiophore and conidia. Conidiophore was fasiculate, arising from stoma, brown, paler towards the apex, 1-4 septate, unbranched (Plate 11). Conidia borne singly at the tip of the conidiophores, cylindrical, filiform, hyaline, smooth, thin walled, multiseptate tapered towards the apex and found wider at the base (Plate12).

Fungus isolated from the collar region of the infected plants was identified as *Rhizoctonia solani* (Plate 13) by observing its mycelial characters such as 90° branching of hyphae (Plate 16), more than 3 nuclei per hyphal cell etc. Sclerotial characters were also studied and found that sclerotia are irregular in shape, at first white in colour, but later turning brown, varying in size from 2 mm to 6 mm. (Plate 15).

Highest relative frequency of *R. solani* (30 per cent) was found in samples collected from Nagaon and Sonitpur districts followed by Jorhat (25 per cent) and Golaghat (20 per cent) district (Table 2). Similarly, highest frequency of *Cercospora* sp. (60 per cent) was prevalent in samples collected from Nagaon district followed by Jorhat (35 per cent) and Sonitpur (35 per cent) districts. *Fusarium* sp. was highly prevalent in Sonitpur (45 per cent) district, followed by Nagaon (40 per cent), Jorhat (20 per cent) and Golaghat (10 percent) district (Table 2). Previous reports says that *Fusarium* sp. was found to be the most prominent among the fungi causing root rot diseases in patchouli which leads to severe damage in patchouli (Sreedevi, 2009 and Zaman, 2009).

Some other insect pests were also recorded in root-knot infested patchouli viz., Leaf Roller (*Pachyzacia* sp.) (Plate 17) and Grass hopper (*Hieroglyphus* sp.) (Plate 18). These insect pests were found to be voracious feeder and almost eat up the green foliage leading to heavy yield loss. Kalita *et al.* (2011) also reported that Patchouli is often attacked by leaf folder, leaf roller and leaf eating grasshoppers.

Conclusion

This research work revealed the abundance of root knot nematode (*Meloidogyne incognita*) in patchouli growing areas of Golaghat, Nagaon, Jorhat and Sonitpur districts of Assam. Besides this different diseases and pests were also associated with root knot nematode forming a complex type of diseased condition in patchouli. Root-knot infested plants seems to be more susceptible to fungal attack then a healthy plant which explains the phenomenon that, nematodes being an primary incitant opens up the pathway for secondary organisms that makes their way to the patchouli roots and aggravetes the disease leading to early death of the host plant. Identification of nematode hot spots in patchouli growing areas and timely management of nematodes will obviously put a check in the multiplication of nematodes and also help in checking other fungal pathogens associated with it there by protecting the valuable plant.

Acknowledgement

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Table 1. Frequency of occurrence of *Meloidogyne* incognita in patchouli in Golaghat Nagaon, Jorhat and Sonitpur districts

Golaghat district	No. of sample collected	No. of sample with root-knot nematode	% frequency of occurrence
Buralikson	9	4	44.44
Badulipar	5	Nil	0.00
Padumoni	6	1	16.66
Dergaon	7	2	28.57
Bhaboligaon	5	Nil	0.00
Total	32	7	21.87
Nagaon district			
Koliabor	15	9	60.00
Ulonigaon	3	1	33.33
Seujnagar	6	2	33.33
Hatigaon	5	Nil	0.00

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Missamukh	7	2	28.57
Total	36	14	38.88
Jorhat district			
Titabor	10	4	40.00
Kumargaon	4	2	50.00
Gajparia gaon	4	Nil	0.00
Bongalgaon	6	1	16.66
Deogharia gaon	4	Nil	0.00
Total	28	7	25.00
Sonitpur district			
Massgaon	8	3	37.50
Madhupur	6	2	33.33
Bordubi	4	2	50.00
Bhandari vill	4	Nil	0.00
Bijoypur	5	2	40.00
Total	27	9	33.33

Table 2. Frequency of occurrence of different fungal pathogens in patchouli in Golaghat, Nagaon, Jorhat and Sonitpur district

Place	Disease	Total no of samples	Infected sample	% frequency occurred
Golaghat	*Cercospora sp.*	20	3	15.00
	Rhizoctonia solani	20	4	20.00
	Fusarium sp.	20	2	10.00
Nagaon	*Cercospora sp.*	20	12	60.00
	Rhizoctonia solani	20	6	30.00
	Fusarium sp.	20	8	40.00
Jorhat	Cercospora sp.	20	7	35.00
	Rhizoctonia solani	20	5	25.00
	Fusarium sp.	20	4	20.00
Sonitpur	Cercospora sp.	20	7	35.00
	Rhizoctonia solani	20	6	30.00
	Fusarium sp.	20	9	45.00

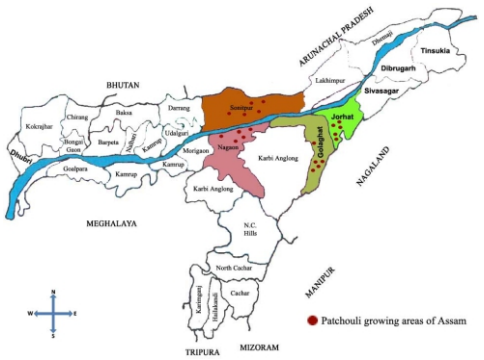

Plate 1. Map showing surveyed districts of Assam

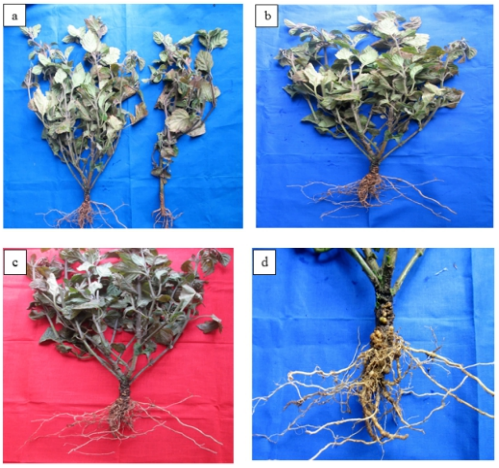

Plate 2. a, b, c, d. *Meloidogyne* incognita infested patchouli

Plate 3. Symptoms of root knot nematode infestation

Plate 4. Perennial pattern of *Meloidogyne* incognita

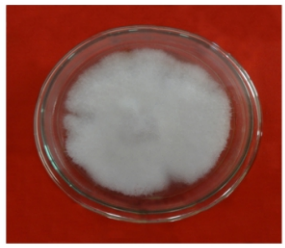

Plate: 5. Pure culture of *Fusarium sp.*

Plate: 6. Roots of wilted plants

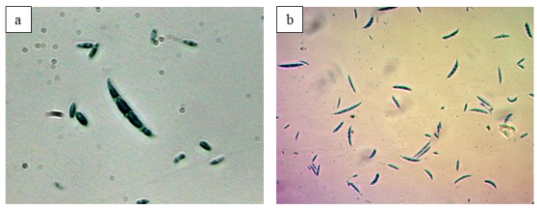

Plate: 7. a, b. Micro and macro conidia of *Fusarium* sp.

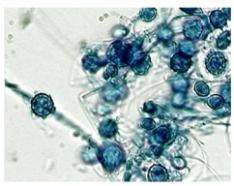

Plate: 8. Clamydospore of *Fusarium* sp.

Plate: 9. Pure culture of *Cercospora* sp.

Plate: 10. Leaf spot caused by *Cercospora* sp.

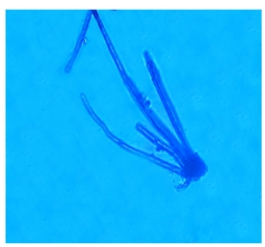

Plate: 11. Conidiophore of *Cercospora* sp.

Plate: 12. Conidia of *Cercospora* sp.

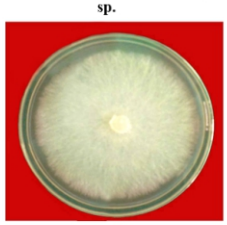

Plate: 13. Pure culture of of *Rhizoctonia solani*

Plate: 14. Collar region of wilted plant

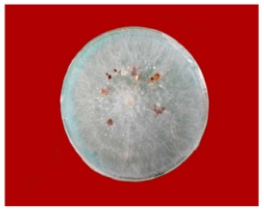

Plate: 15. Mycelial growth with sclerotia of *Rhizoctonia solani* on PDA

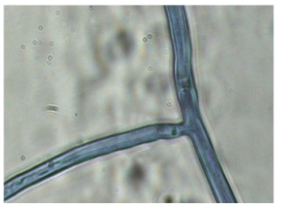

Plate: 16. Mycelium of *Rhizoctonia solani*

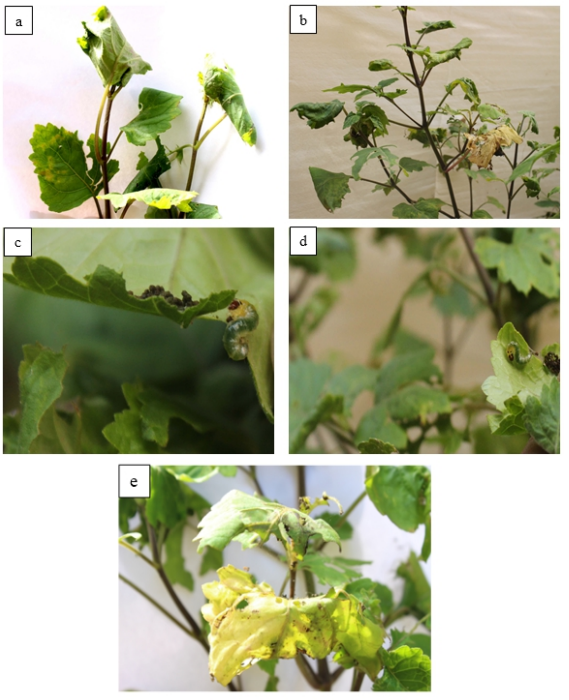

Plate 17. a, b, c, d, e. Leaf roller infestation in patchouli

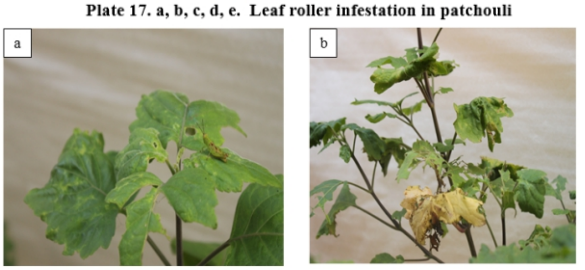

Plate 18 a, b. Grasshopper infestation in patchouli

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