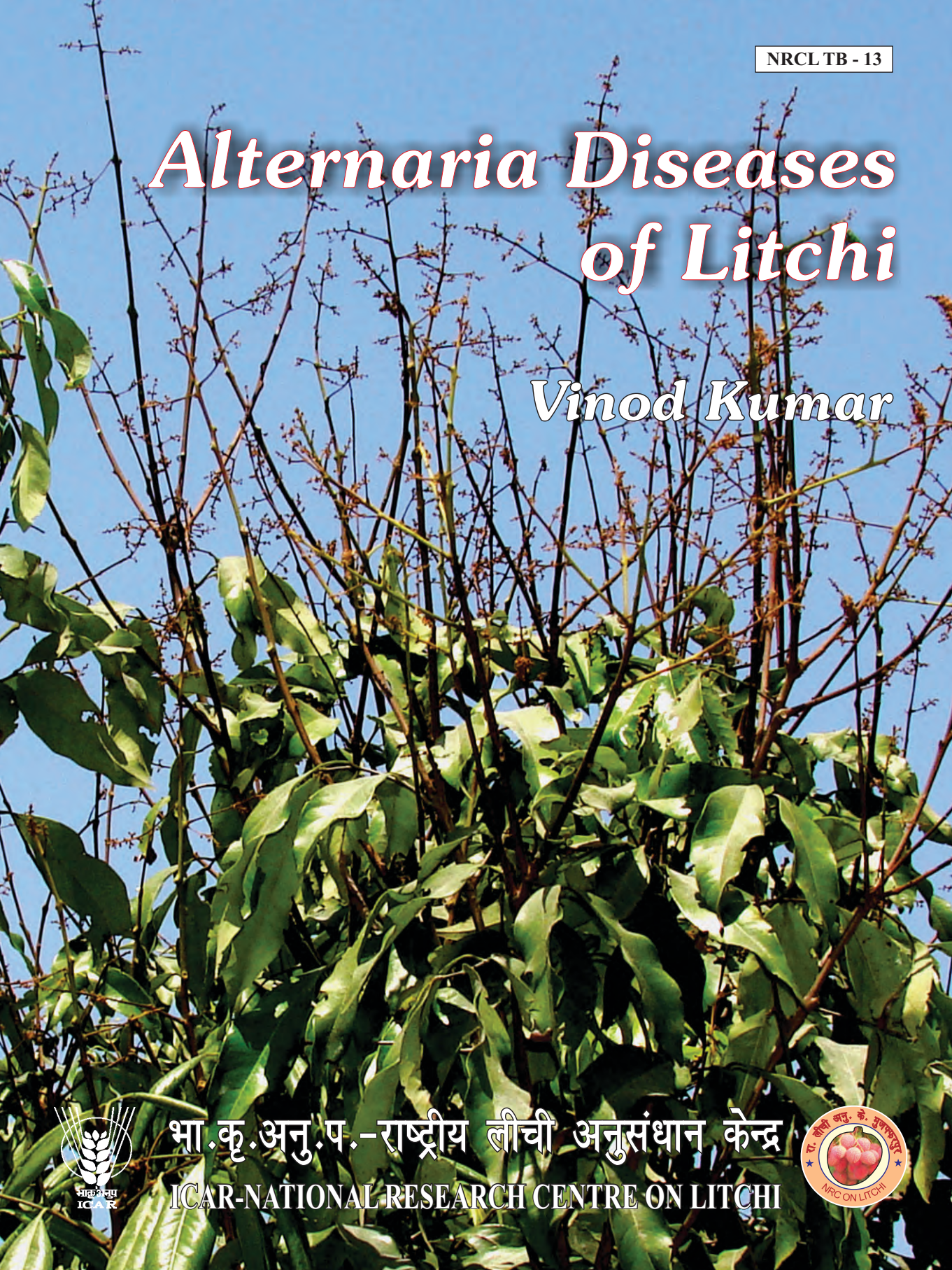
NRCL TB - 13

Alternaria Diseases of Litchi

Vinod Kumar



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ICAR-NATIONAL RESEARCH CENTRE ON LITCHI

Abstract

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Litchi as compared to many fruit bearing trees is less affected by diseases in India. However, co-evolution of pathogens with changing climate and cropping system over the years has affected disease dynamics in litchi ecosystem. *Alternaria alternata* has emerged as the most important pathogen of litchi causing disease at three phase *viz.,* leaf blight on nursery plants and orchard trees in vegetative phase, panicle blight and fruit blight in reproductive phase, and fruit rot at post-harvest. Leaf blight was first noticed during 2012 on nursery plants. Blights of panicle and fruit were first noticed during April-June, 2014. At post-harvest stages, it is a dominant pathogen causing fruit decay. *A. alternata* have specialized itself in pathogenic capability, evolving itself from saprophyte to a specialized necrotrophic pathogen. In a scenario of rapid climate changes, the tolerance demonstrated by *A. alternata,* which is able to survive in prolonged stressing conditions of high and low temperature and humidity regime, increase the concern about this pathogen expecting higher levels of occurrence. This bulletin summarizes current state of knowledge about diseases caused by *A. alternata* in litchi, symptoms, aetiology, basic characteristics of the pathogen, pathogenic variability, incidence and severity of the disease, and epidemiology which is the outcome of studies conducted at NRCL Muzaffarpur. Various options for disease management are discussed and an integrated approach is advocated.

सारांश

कई फल देने वाले पेड़ों की तुलना में लीची भारत में रोगों से कम प्रभावित होती है। हालांकि, हाल के वर्षों में बदलती जलवायु और फसल प्रणाली के साथ रोगजनकों के सह-विकास ने लीची पारिस्थितिकी तंत्र में रोग की गतिशीलता को प्रभावित किया है। *अल्टरनेरिया अल्टरनेटा* लीची के सबसे महत्वपूर्ण रोगजनक के रूप में उभरा है, जो तीन चरण में रोग पैदा करता है। 'वानस्पतिक अवस्था' में नर्सरी के पौधों और बाग के पेड़ की पत्तियों का झुलस जाना, 'फलन अवस्था' में मंजर और फल का झुलस जाना, और 'तुड़ाई-उपरांत अवस्था' में फल सड़न का होना। पत्ती झुलसा रोग पहली बार 2012 में नर्सरी पौधों में देखा गया था। अप्रैल-जून, 2014 के दौरान पहली बार 'मंजर' और 'फल' का झुलसा रोग देखा गया था। यह तुड़ाई-उपरांत फल सड़न पैदा करने के लिये एक मुख्य रोगजनक पहले से ही है। मृतजीवी कवक से विशिष्ट नेक्रोट्रोफिक रोगजनक के रूप में *अल्टरनेरिया अल्टरनेटा* ने स्वयं को विकसित कर लिया है। तेजी से हो रहे जलवायु परिवर्तन के परिदृश्य में *अल्टरनेरिया अल्टरनेटा* द्वारा प्रदर्शित सहिष्णुता, जो उच्च और निम्न तापमान के साथ-साथ अधिकतम से न्यूनतम नमी वाली तनावपूर्ण परिस्थितियों में भी लंबे समय तक जीवित रहने में सक्षम है, इस रोगजनक के संभावित फैलाव और होने वाली हानि के बारे में चिंता को बढ़ाता है। यह तकनीकी बुलेटिन *अल्टरनेरिया अल्टरनेटा* द्वारा लीची में होनेवाले रोगों, लक्षण, उनके हेतु विज्ञान (एटिओलॉजी), रोगजनक की बुनियादी प्रकृति, रोगजनक परिवर्तनशीलता, रोग की व्यापकता और तीव्रता, महामारी के लिये अनुकूल मौसम और कारकों के बारे में ज्ञान की वर्तमान स्थिति का सारांश देता है, जो एनआरसीएल, मुजफ्फरपुर में किये गए अध्ययनों का परिणाम है। इसमें रोग प्रबंधन के विभिन्न विकल्पों पर चर्चा के साथ-साथ एकीकृत प्रबंधन की सिफारिश की गई है।

Cover: Symptoms of panicle blight of litchi

Alternaria Diseases of Litchi

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Published by

Dr. Vishal Nath, Director, ICAR- National Research Centre on Litchi, Muzaffarpur

Contents

Preface

Litchi is a subtropical evergreen fruit tree extensively grown in China, India, Thailand, Vietnam and the rest of tropical Southeast Asia, the Indian Subcontinent. It is world-famous for its attractive colour, taste and quality. Major litchi producing states in India are Bihar, West Bengal, Assam and Jharkhand. The demand for litchi fruits and its products continues to increase in both domestic and overseas markets. Though India is second largest producer of litchi in the world, productivity is low. One of the many reasons for this is non adoption of proper orchard husbandry practices including management of pests and diseases.

Litchi is less affected by diseases than many other fruit trees in India. However, co-evolution of pathogens with changing climate and cropping system over the years has affected disease dynamics in litchi ecosystem. During recent years, *Alternaria alternata* has emerged as the most important pathogen of litchi causing disease at multiple phenophases (vegetative, reproductive and post harvest) of litchi. *A. alternata* have specialized itself in pathogenic capability, evolving itself from saprophyte to a specialized necrotrophic pathogen. *A. alternata* can very well tolerate prolonged stressing conditions and is able to survive through high and low temperature and humidity regime. Litchi is cultivated over a large area in Bihar and adjoining states of India. Considering the pathogenic capability of *A. alternata* and availability of a large host population, higher levels of occurrence of disease is expected.

Correct diagnosis is important for the management of diseases. This bulletin presents the current knowledge of *Alternaria* diseases of litchi including symptomatology, aetiology, basic characteristics of the pathogen, pathogenic variability, incidence and severity of the disease, and epidemiology which is the outcome of studies conducted at NRCL Muzaffarpur. Various options for disease management are discussed. I hope this publication will be useful for researchers and extension personnel who may not have ready access to specialized journals and reports. It should be of value to teachers and students of horticulture as well.

Vinod Kumar

Alternaria Diseases of Litchi

Introduction

Litchi or lychee (*Litchi chinensis* Sonn.) [Family Sapindaceae] is a tropical and subtropical fruit tree native to the Guangdong and Fujian provinces of China, and now cultivated in many parts of the world. Litchi is extensively grown in China, India, Thailand, Vietnam and the rest of tropical Southeast Asia, the Indian Subcontinent (Papademetriou and Dent 2002), and more recently in South Africa, Brazil, the Caribbean, Queensland, California and Florida (Crane et al. 2008). India and China account for 91 % of the world litchi production. The acreage under litchi cultivation in India was 90,000 ha with a production of 5,59,000 tonnes during 2015-16 (NHB 2017). Major litchi producing states in India are Bihar, West Bengal, Assam and Jharkhand (Fig.1). Bihar contributes 45 % of total litchi production and has 40 % of the acreage (Kumar et al. 2014). Litchi is a popular fruit due to its distinctive taste, pleasant flavour and appealing pinkish-red colour. Fruits are rich in vitamin C, niacin, riboflavin, thiamine, folate and β-carotene. They also contain minerals such as potassium, phosphorous, calcium, magnesium and copper. These low-calorie fruits contain no saturated fats or cholesterol, but are rich in dietary fibre and polyphenols. The demand for litchi fruits and its products continues to increase in both domestic and overseas markets.

Litchi is less affected by diseases than many other fruit trees in India. Some of the economically important diseases of litchi are leaf spots (*Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. and *Botryodiplodia theobromae Pat.*), anthracnose (*Colletotrichum gloeosporioides*) and twig blight (*C. gloeosporioides* and *Gloeosporium* sp.) at pre-harvest stage (Kumar et al. 2011, 2014). Diseases are more of a postharvest issue, although many of the fruit are infected before harvest. Postharvest fruit rots are caused by several pathogens, including *Alternaria alternata (Fr.) Keissl., Aspergillus flavus Link, Cylindrocarpon tonkinense* Bugn., *Botryodiplodia theobromae* and *C. gloeosporioides* (Prasad and Bilgrami 1974; Awasthi et al. 2005; Kumar et al. 2016a, 2016b). Kumar et al. (2016a) reported *A. alternata* as the most common pathogen causing post-harvest litchi fruit decay in India.

Alternaria alternata is an opportunistic pathogen on a reported 380 host species (Ellis 1971; Farr et al. 1989; Murthy et al. 2003) causing leaf spots, rots and blights on many plant parts. Since last 10 years, *A. alternata* has been reported on several new hots (Table 1). Among fruit crops, it has previously been reported on apple (Johnson et al. 2000), citrus (Peever et al. 2002) and pomegranate (Ezra et al. 2010). It has also been reported to cause a post-harvest fruit decay of litchi fruits in Australia (Johnson et al. 2002), India (Kumar et al. 2016a) and Pakistan (Alam et al. 2017). A post-harvest loss of 35-44 % of litchi fruits has been reported by Kumar et al. (2016a), and much of this is due to decay caused by *A. alternata*.

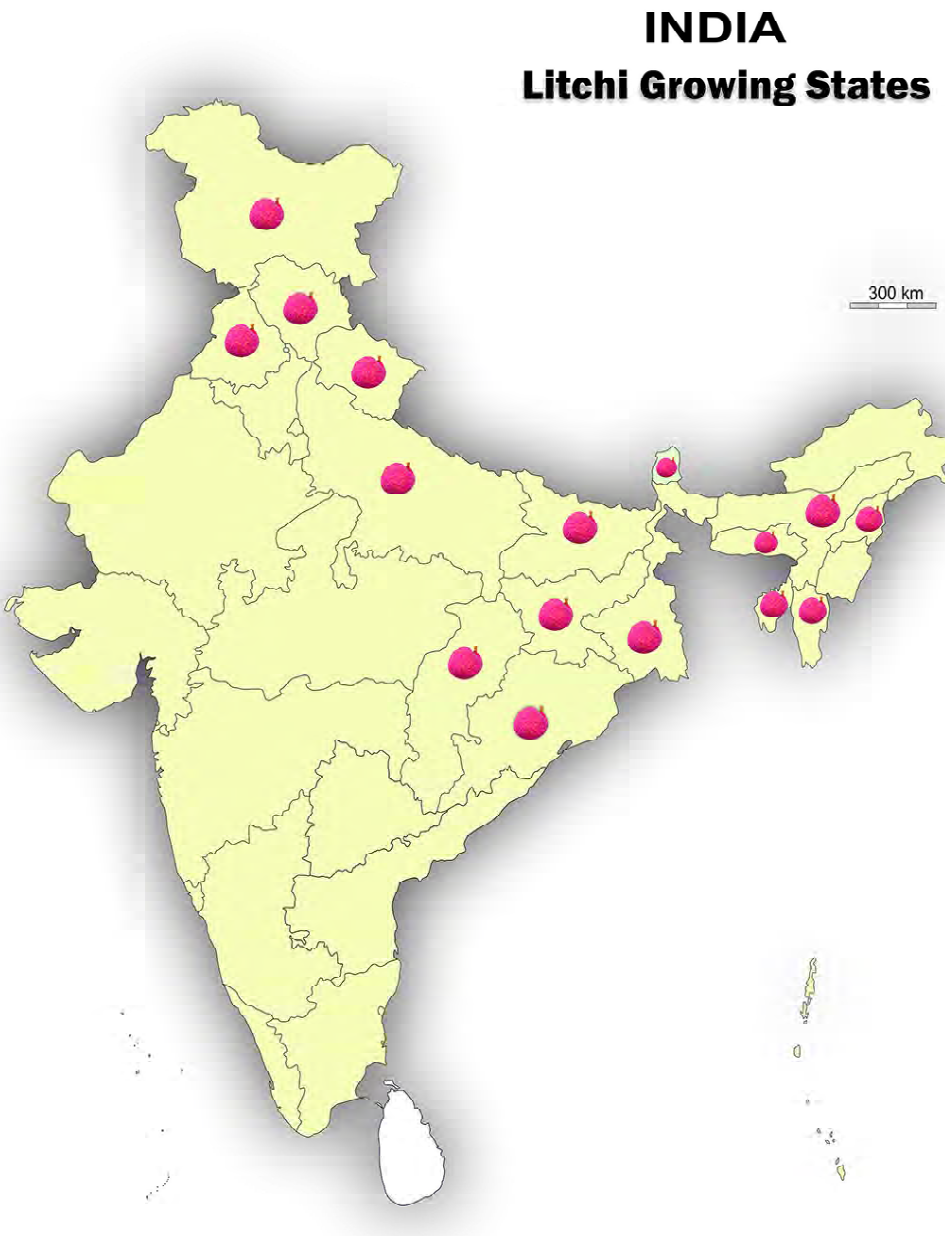

Fig 1. Litchi growing states of India

Table 1. Occurrence of *Alternaria alternata* on different host plants reported since 2008

Pathogen/ *Alternaria* sp.	Host		Disease	Reference	Remarks
	Common name	Scientific name			
Alternaria alternata	Litchi or Lychee	*Litchi chinensis*	Leaf, Panicle and fruit blight	Kumar *et al.*, 2018	First report, Bihar, India
Alternaria alternata	Strawberry	*Fragaria × ananassa*	Leaf spot	Mehmood *et al*.,2018	First report, Pakistan
Alternaria alternata	Blue honeysuckle	*Lonicera caerulea var. kamtschatica*	Black spot	Mirzwa-Mróz *et al*.,2018	First report, Poland
Alternaria alternata	Lychee	*Litchi chinensis*	Postharvest fruit Rot	Alam *et al*.,2017	First Report, Pakistan
Alternaria alternata	Ethiopian mustard	*Brassica carinata*	Black spot	Dunbar *et al*.,2017	First report, South Dakota
Alternaria alternata	Manoranjini	*Artabotrys hexapetalus*	Leaf blight	Kumar and Singh, 2016	Uttar Pradesh, India
Alternaria alternata	Ashok, False Ashok	*Polyalthia longifolia*	Leaf spot	Akhtar *et al*., 2016	First report, Pakistan
Alternaria species	Hazelnut	*Corylus avellana*	Blight	Prashad *et al*.,2016	First report, India
Alternaria alternata	Sorghum	*Sorghum bicolor*	Leaf spot	Zhao *et al*.,2016	First report, China
Alternaria alternata	Jujube	*Zizyphus jujuba*	Brown spot	Bai *et al*., 2015	First report, China
Alternaria alternata	Jujube	*Zizyphus jujuba*	Leaf spot	Bai *et al*., 2015	First report, China
Alternaria alternata	Onion	*Allium cepa*	Leaf blight	Bihon *et al*.,2015	First report, South Africa
Alternaria alternata	Rubber tree	*Hevea brasiliensis*	Black leaf spot	Cai *et al*.,2015	First report, China
Alternaria alternata	Spinach	*Spinacia oleracea*	Leaf spot	Czajka *et al*.,2015	First report, Poland
Alternaria alternata	Pomegranate	*Punica granatum*	Heart rot	Faedda *et al*.,2015	First report, Italy
Alternaria alternata	Tumu Merah	*Bruguiera gymnorrhiza*	Leaf spot	Lin *et al*.,2015	First report, China
Alternaria alternata	Agave	*Agave americana*	Leaf spot	Mirhosseini *et al*.,2015	First record, Iran
Alternaria alternata	Chinese dwarf banana	*Ensete lasiocarpum*	Leaf spot	Fu *et al*.,2014	First report, China
Alternaria alternata	Apple	*Malus – domestica* Borkh	Postharvest decay	Jurick *et al*.,2014	First report, Pennsylvania
Alternaria alternata	Euphrates poplar or Desert poplar	*Populus euphratica*	Leaf spot	Osdaghi *et al*.,2014	First report, Iran
Alternaria alternata	White goosefoot /Bathua	*Chenopodium album*	Leaf blight	Patel *et al*., 2014	First report, India

Alternaria alternata	Peppermint	*Mentha piperita*	Leaf spot	Zarandi *et al*.,2014	First report, Iran
Alternaria alternata	Tea	*Camellia sinensis*	Leaf spots	Zhou and Xu , 2014	First report, China
Alternaria alternata	Date palm	*Phoenix dactylifera*	Postharvest black spot	Palou *et al*.,2013	First report, Spain
Alternaria alternata	Ribben plant	*Zizyphus jujuba*	Leaf blight	Muthukumar and Venkatesh, 2013	A new record, India
Alternaria alternata	Banana	*Musa* spp	Leaf spot	Parkunan *et al*.,2013	First report, United States
Alternaria alternata	Sweet persimmon fruits	*Diospyros kaki*	Black spot	JungHan *et al*., 2013	First report
Alternaria	Okra	*Hibiscus esculentus*	Pod blight	Ziedan , 2012	First report, Egypt
Alternaria alternata	Persimmon	*Diospyros kaki*	Postharvest black spot	Palou *et al*.,2012	First report, Spain
Alternaria alternata	Cherry fruits	*Cerasus pseudocerasus*	Black spot	Zhao and Liu, 2012	First report, China
Alternaria alternata	Jatropha	*Jatropha curcas*	Inflorescence blight	Espinoza-Verduzco Mde los *et al*., 2012	First report, Sinaloa, Mexico
Alternaria	Gerbera	*Gerbera jamesonii*	Leaf spot	Farhood and Hadian, 2012	First report, north of Iran
Alternaria alternata	Kiwifruit	*Actinidia deliciosa*	-	Karakaya and Çelik , 2012	First report, Turkey
Alternaria alternata	Spinach	*Spinacia oleracea*	Leaf spots	Marraiki *et al*.,2012	First report, Saudi Arabia
Alternaria alternata	Amaranthus seeds	*Amaranthus mantegazzianus* syn. *A. caudatus subsp. mantegazzianus*	Discoloration	Noelting *et al*.,2011	First report, Argentina
Alternaria alternata	Golden crown beard	*Verbesina encelioides*	-	Perveen and Bokhari, 2011	First report Saudi Arabia
Alternaria alternata	Indian spinach	*Basella alba*	Leaf blight	Sankar *et al*.,2011	First report, India
Alternaria alternata	Fish mint	*Houttuynia cordata*	Leaf spot	Zheng *et al*., 2011	First report, China
Alternaria alternata	Sweet basil	*Ocimum basilicum*	Leaf spot	Garibaldi *et al*.,2011	First report, Italy
Alternaria alternata	Citrus	*Citrus reticulata*	Brown spot	Wang *et al*.,2010	First report, China
Alternaria alternata	Pomegranate	*Punica granatum*	Fruit rot	Tziros *et al*.,2008	Greece

A leaf blight disease was observed during May 2012 on potted litchi plants in nurseries at Muzaffarpur, Bihar, and subsequently, leaf blight was observed on adult trees in litchi orchards. The occurrence of panicle/inflorescence and fruit blights was first noticed during April to June, 2014 at National Research Centre on Litchi (NRCL), Muzaffarpur Experimental Farm. In subsequent years, blights of leaves, panicle/inflorescence and fruits were commonly observed on orchard trees in Bihar and adjoining states of India which was reported to be caused by *A. alternata* (Kumar et al. 2018; Kumar and Anal 2018). The cultivation of litchi over a large area in Bihar and adjoining states of India provides the pathogen with the opportunity to spread and cause heavy crop loss. Thus, considering cumulative loss incurred at different stages of the crop and in supply chain, detail studies was carried out, results of which are presented in this bulletin.

Symptoms and occurrence

A leaf blight disease affected a significant number of litchi plants in nurseries at the NRCL Experimental Farm, Bihar, India in May 2012 (Figs. 2 a, b) and caused heavy losses. Initial symptoms resembled potassium deficiency and started from the leaf tips as a light to dark brown necrosis that advanced towards the margins, leading to complete necrosis and drying of the affected leaves. On orchard trees, leaf blight similar to that on nursery plants was observed. The leaf blight was more damaging to nursery plants and new orchard plantings in the early establishment phase where it drastically hampered growth of plants as compared to mature orchard trees. Initially, senescing leaves were infected but with the progression of the disease, all the leaves became blighted except a few upper leaves. The leaf blight was not economically important in adult bearing trees as the pathogen was mostly limited to old senescing leaves. During flowering and fruit development, blighting of panicles and fruits occurred. Panicles shriveled and dried up as a result of necrosis (Fig. 2c), while necrosis of the pedicel led to complete drying of the rind of developing fruits (Fig. 2d). Fruit blight developed only when the pedicel was infected by the pathogen, and if fruit were infected, small black lesions developed on the rinds which later caused post-harvest fruit decay. Major losses were the result of infection of panicles leading to partial blighting of panicles on portions of trees. Most of the trees surveyed in orchards had less than 20 % diseased panicles, but there was, nevertheless, heavy loss to the crop. Fruit blight incidence was sporadic in orchards and only 5-10 % of the fruit were affected. Fruit decay/rots usually develop in supply chain at retail level. Symptoms are initially perceptible on injured portion of fruits. The decayed areas get depressed and rot gradually penetrates deep into the pulp (Fig. 2e). Fruits emit an odour of fermentation.

Fig. 2a. Symptoms of leaf blight on nursery plants

Fig. 2b. Symptoms of leaf blight on an orchard tree

Fig. 2c. Symptoms of panicle blight. Left- Healthy panicles with fruit set, Right- Diseased panicles with no fruit set

Fig. 2d. Symptoms of fruit blight. Left- in the field, Right - Diseased fruits

Fig. 2e. Post harvest fruit rots/decay caused by *Alternaria alternata*

Isolation of pathogen

Symptomatic leaves were collected from 10 infected plants from six nurseries, and from two orchard trees of litchi at the NRCL Experimental Farm, Bihar in May 2012. Similarly, six diseased panicle and six diseased fruit specimens were collected in April and June, 2014, respectively, from trees in orchards located at Mushahari, Muzaffarpur. Pathogens causing fruit decay were also isolated on potato dextrose agar medium (PDA). Fungal isolations were made by surface-disinfesting small fragments of symptomatic leaf, panicle and fruit tissues in 0.5% NaOCl, double-rinsing in sterile water, and plating onto PDA amended with 0.05 g/L streptomycin sulphate. Dishes were incubated at 28 ± 1 °C for six days and pure cultures were obtained using the hyphal tip method. The frequency of recovery of isolates from plated tissues was 100%. The isolates were maintained on PDA slants.

Morphological identification

Microscopic examinations were conducted by mounting fungal tissues in water and lactophenol, and dimensions of 30 conidia per culture were measured from six-day old cultures. Initial identification of the fungi isolated from diseased tissues collected in the field was made on the basis of morphology and growth characteristics on PDA. Dimensions of the conidia and conidiophores were measured using ocular and stage micrometer mounted on a light microscope (Nikon Eclipse 50*i*).

Typical fungal colonies isolated from the diseased litchi tissues on PDA were olive green in colour, having a prominent 2-6 mm white margin (Fig. 3). A total of 24 isolates (12 from leaves, 6 from panicles and 6 from fruits) were obtained from diseased tissues. They grew to 43.5-81.5 mm

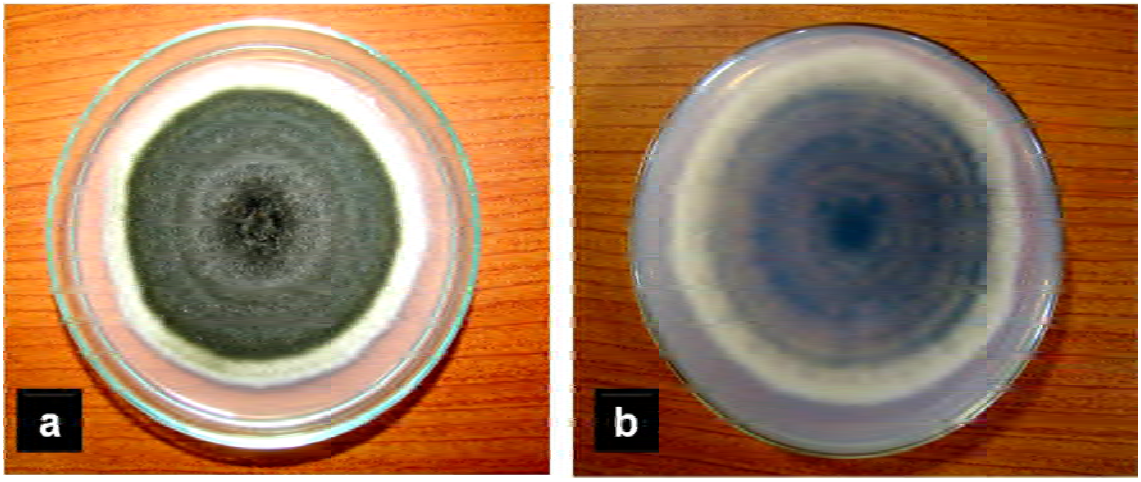

Fig. 3. *Alternaria alternata*: a. Top view of colony on PDA medium,
b. Reverse view of colony on PDA medium

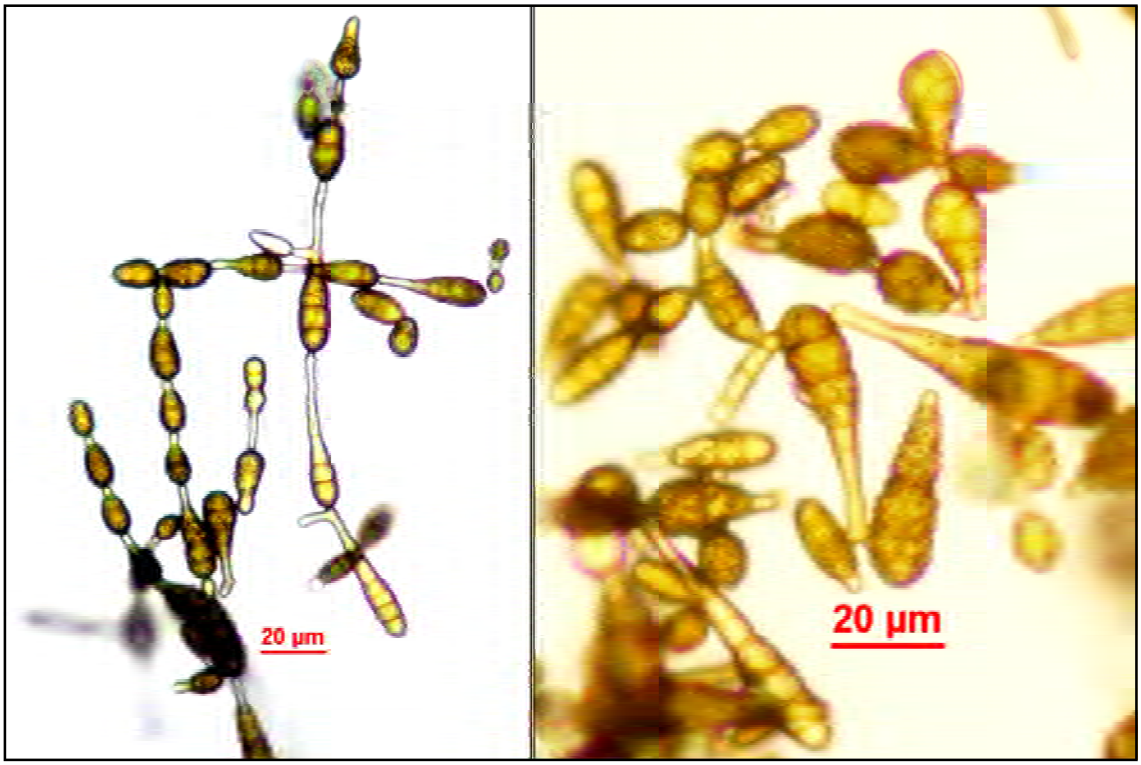

Fig. 4. *Alternaria alternata*: Left- Conidia in chains,
Right- Variation in conidia (note the surface architecture)

in diameter after 6 days. The branched, brownish, septate mycelia produced dark brown, obclavate to obpyriform, catenulate conidia on short conidiophores (Fig. 4). The number of conidia in chains varied from 6 to 14 with secondary chains having 3 to 8 conidia. Conidia (n=30) were 16 to 40 µm long (avg. 29.8; SD ± 4.3 µm), 4 to 13 µm wide in the broadest part (avg. 7.6; SD ± 2.6 µm) with a beak 0 to 10 µm long (avg. 4.4; SD ± 2.7 µm), and 1 to 4 transverse and 0 to 3 longitudinal or oblique septa. These morphological characteristics and measurements match those of *A. alternata* (Ellis 1971; Simmons 2007) and hence the isolated fungi were identified as *Alternaria alternata* (Fr.) Keissler. The conidial surface of all the isolates was delicately pitted rather than smooth-walled, unlike typical *A. alternata* strains. Among the leaf blight isolates, three distinct strains designated as AA-L_1, AA-L_2, AA-L_3, were identified based on variation in cultural characteristics on PDA. The morpho-cultural characteristics of all the panicle blight and the fruit blight isolates of the fungus resembled the strain AA-L_3.

Pathogenic variability

Distinct variability in pathogen was observed infecting different parts at different phenophases of litchi. Three leaf blight strain/pathotype (AA-L_1, AA-L_2 and AA-L_3) (Fig. 5, Table 2), two panicle blight pathotype (AA-P_1, AA-P_2) (Fig. 6, 7), one fruit blight pathotype (AA-F_2) (Fig. 9) and two fruit decay pathotype (AA-F_1, AA-F_3) (Fig. 8, 10) were identified, some of which were submitted to Genbank. Cross infectivity of the three leaf blight pathotypes for panicles and fruit infection was observed with varying severity, and conversely panicle and fruit blight pathotype caused leaf infection. Characteristics of leaf blight pathotypes were as under:

Pathotype AA-L_1 (GenBank accession no KR149264): Symptoms appeared as leaf necrosis starting from tip of leaf lamina, colour of necrotic area light brown. Colony on PDA slow growing, irregular, dark green with white margin, concentric growth rings absent, reverse view appeared blackish without growth rings, dark pink pigmentation; conidia oval to ellipsoidal, mean conidial dimension 29.8 ± 4.3 µm with 2-6 transverse and 1-3 longitudinal or oblique septa, tapering gradually to form a short swollen beak at the apex; conidial length 16-40 µm, width at broadest part 4-13 µm and beak 0-10 µm.

Pathotype AA-L_2 (GenBank accession no KR149265): Symptoms appeared as leaf necrosis starting from tip that advanced very fast leading to complete or partial blighting and scorching of leaves. The affected leaves soon withered and dropped off. Colony on PDA moderately fast growing compared to other two pathotypes, circular with smooth margin, front colour olivaceous green with smooth, white margin (resembling to typical colony of *Aspergillus flavus*), reverse light pinkish without growth rings; conidia obclavate to oval, mean conidial dimension 21.4 ± 3.8 µm with 1-4 transverse and 0-3 longitudinal or oblique septa, tapering gradually to form a short swollen beak at the apex; conidial length 12-38 µm, width at broadest part 4-12 ìm and beak 0-6 µm. More number of shorter conidia was formed compared to pathotype AA-L_2.

Pathotype AA-L_3 (GenBank accession no KR149266): Symptoms appeared as marginal leaf necrosis that may or may not start from tip of the leaf lamina, colour of necrotic area dark brown, advanced to cause marginal blighting of lamina. Colony on PDA fast growing, circular with white smooth margin, front view greenish black with concentric growth rings, reverse view blackish with concentric growth rings; conidia oval to ellipsoidal, conidia formed in long, often branched chains, mean conidial dimension 34.5 ± 5.6 µm with 3-8 transverse and 2-4 longitudinal or oblique septa, tapering gradually to form a short swollen beak at the apex; conidial length 15-49 µm, width at broadest part 6-16 µm and beak 0-14 µm.

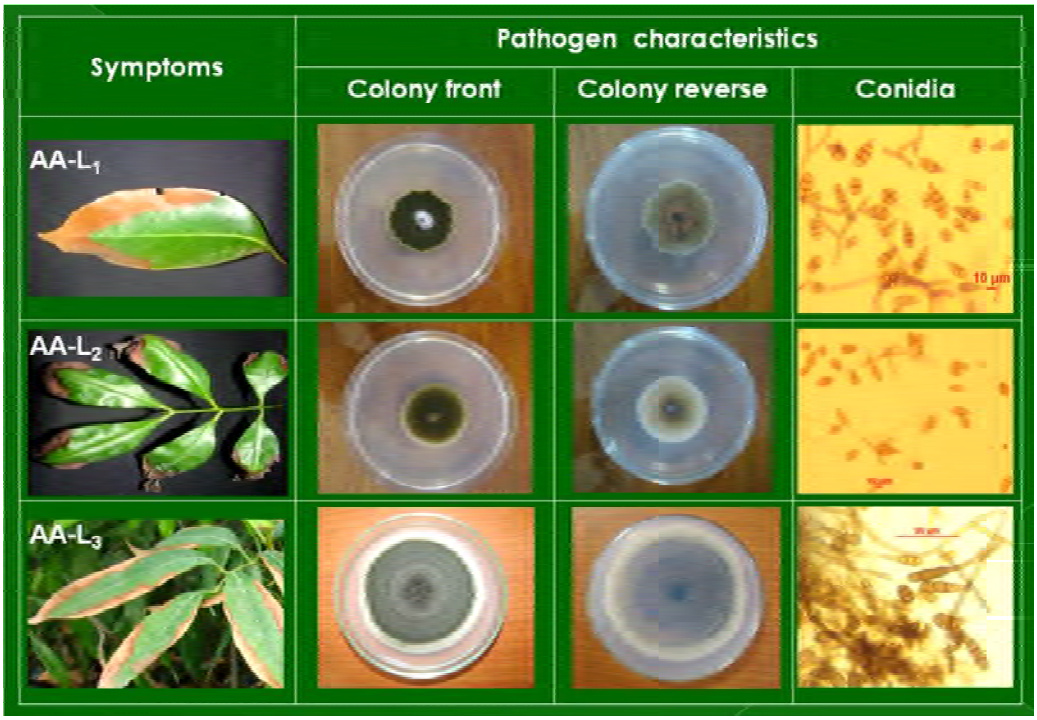

Fig. 5. Variability and characteristics of leaf blight strains of *Alternaria alternata*

Table 2. Dimension and characteristics of litchi leaf isolates of *Alternaria alternata* from Bihar

Pathotype	Conidial Body						Conidial Septation	
	Length (µm)		Width (µm)		Beak Length (µm)		Transverse	Longitudinal
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD		
AA-L_1	16-40	29.8±4.3	4-13	7.6±2.6	0-10	4.4±2.7	2-6	1-3
AA-L_2	12-38	21.4±3.8	4-12	5.4±3.3	0-6	3.0±2.2	1-4	0-3
AA-L_3	15-49	34.5±5.6	6-16	12.2±3.0	0-14	6.3±4.1	3-8	2-4

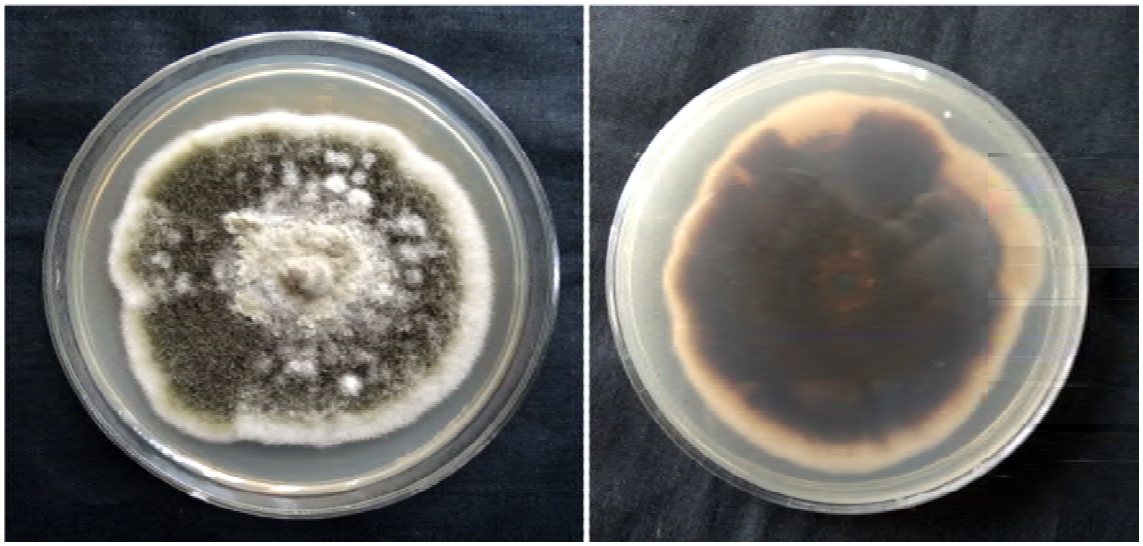

Fig. 6. *Alternaria alternata* pathotype AA-P_1 causing panicle blight

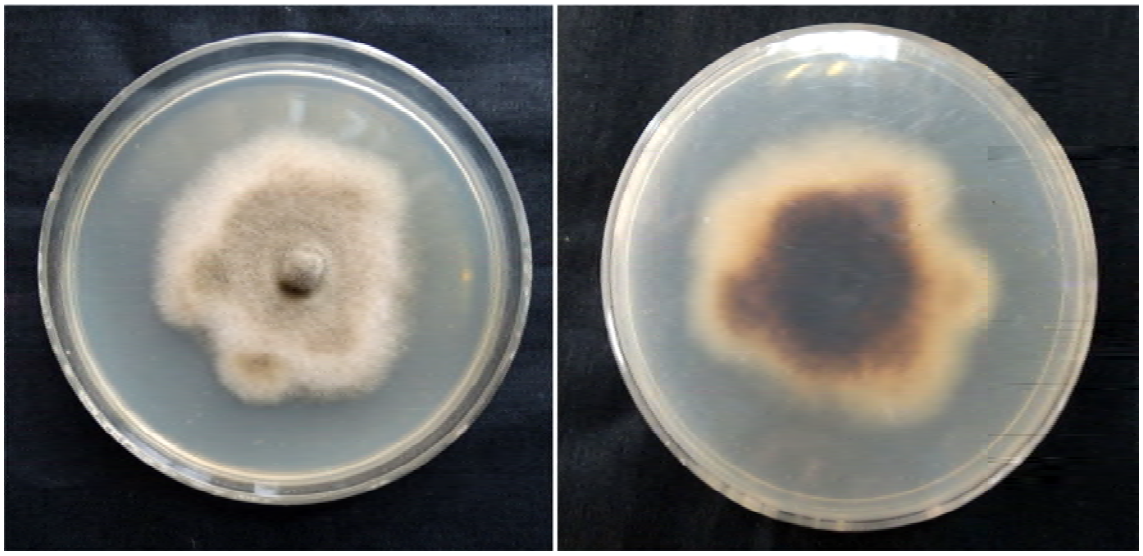

Fig. 7. *Alternaria alternata* pathotype AA-P_2 causing panicle blight

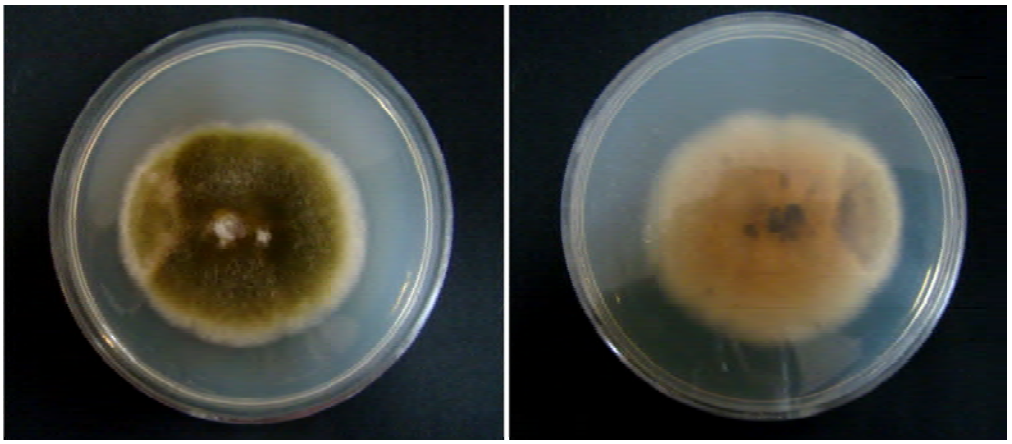

Fig. 8. *Alternaria alternata* pathotype AA-F_1 causing fruit rots/decay

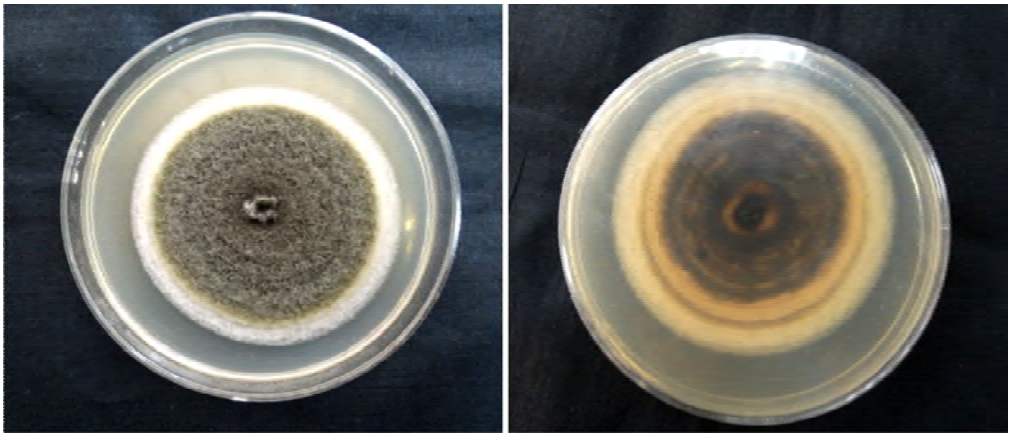

Fig. 9. *Alternaria alternata* pathotype AA-F_2 causing fruit blight

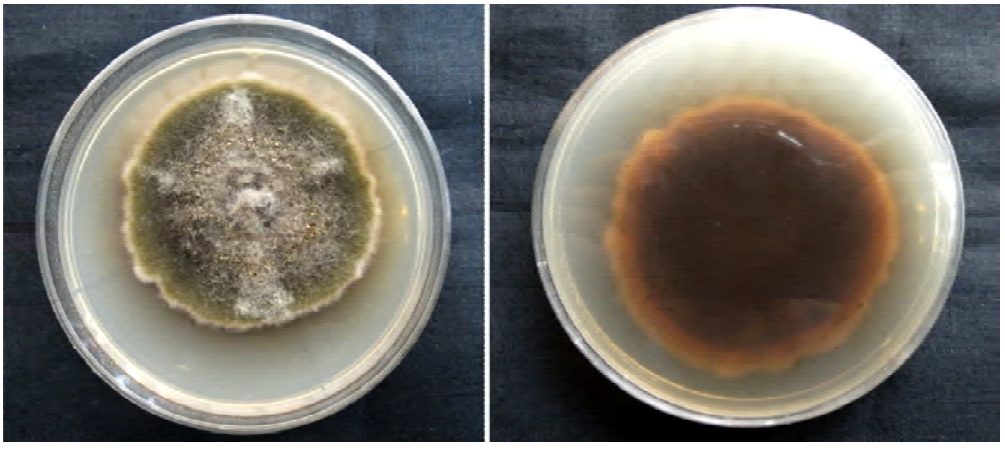

Fig. 10. *Alternaria alternata* pathotype AA-F_3 causing black blemishes on rind of fruit before ripening

Frequency of association of *A. alternata* with blighted panicle

From each orchard, 20 different samples of blighted panicle were collected. A bit of infected tissue along with healthy portion was taken from the specimens and rinsed with distilled water followed by surface sterilization with 1 % NaOCl for 2 min and finally rinsed twice with sterilized distilled water. After surface sterilization, these bits were longitudinally split into two pieces with the help of sterilized blade keeping in a sterile Petri dish under laminar flow. It was then transferred onto PDA plates and incubated at 27 ± 1 ºC. The results showed a degree of association of *A. alternata* with 50.0-92.5 % (mean 74%) with blighted panicle samples (Fig. 11). The remaining had no pathogen growth and might have dried due to physiological or environmental stress.

Fig. 11. Mycelia growth of *Alternaria alternata* on infected bits taken from blighted panicles

Molecular identification

Molecular identification was done by sequencing the internal transcribed spacer (ITS) region of rDNA using primers ITS-1 and ITS-4 (White et al. 1990; Naik et al. 2017) using the dideoxynucleotide chain termination method. Four isolates were grown in potato dextrose broth at 28 ± 1 ºC for seven days. After incubation, the mycelial mats were collected and dried completely on pre-autoclaved filter paper. The dried mycelial mats were ground with liquid nitrogen and DNA was extracted using the CTAB (Cetyltrimethyl ammonium bromide) extraction method (Aradhya et al. 2001). The DNA was amplified with ITS-1 (52-TCCGTAGGTGAACCTGCGG-32) and ITS-4 (52-TCCTCCGCTTATTGATATGC-32) primers. Amplified DNA from PCR was purified using the QIAquick PCR Purification Kit (Qiagen Inc., CA) as specified by the manufacturer and directly cycle sequenced in both directions using the GenomeLab™ Dye Terminator Cycle Sequencing Quick Start Kit on an GenomeLab™ GeXP Genetic Analysis System (Beckman Coulter Inc, CA). The sequence obtained was submitted to NCBI Gen Bank and an accession number was obtained. The sequence analysis was carried out using the BLAST bioinformatics tool of NCBI. Consensus sequences of the ITS1 region of four isolates and reference sequences downloaded

from GenBank were aligned using the multiple sequence alignment program Clustal W and a phylogenetic analysis was performed using the program MEGA version 5 (Tamura et al. 2011). *Bipolaris tetramera* was used as the out-group taxon.

BLAST analysis of sequences showed 100 % homology with several *A. alternata* strains (e.g. KJ008700.1, KY305051.1, KM076936.1, MF785102.1, KR149267.1). The generated sequences of the three leaf blight strains of the pathogen (AA-L_1 - 527 bp, AA-L_2 - 540 bp, AA-L_3 - 567 bp) were submitted to GenBank (accession nos. KR149264, KR149265 and KR149266, respectively). Additionally, a sequence of one fruit decay causing strain (AA-F_1 - 556 bp) of *A. alternata* from litchi was also submitted to GenBank (accession no. KR149267). The isolates were clustered in a distinct clade with those of *A. alternata* isolates retrieved from GenBank with 100 % bootstrap value (Fig. 12). These results confirmed the identity of the fungus as *A. alternata*.

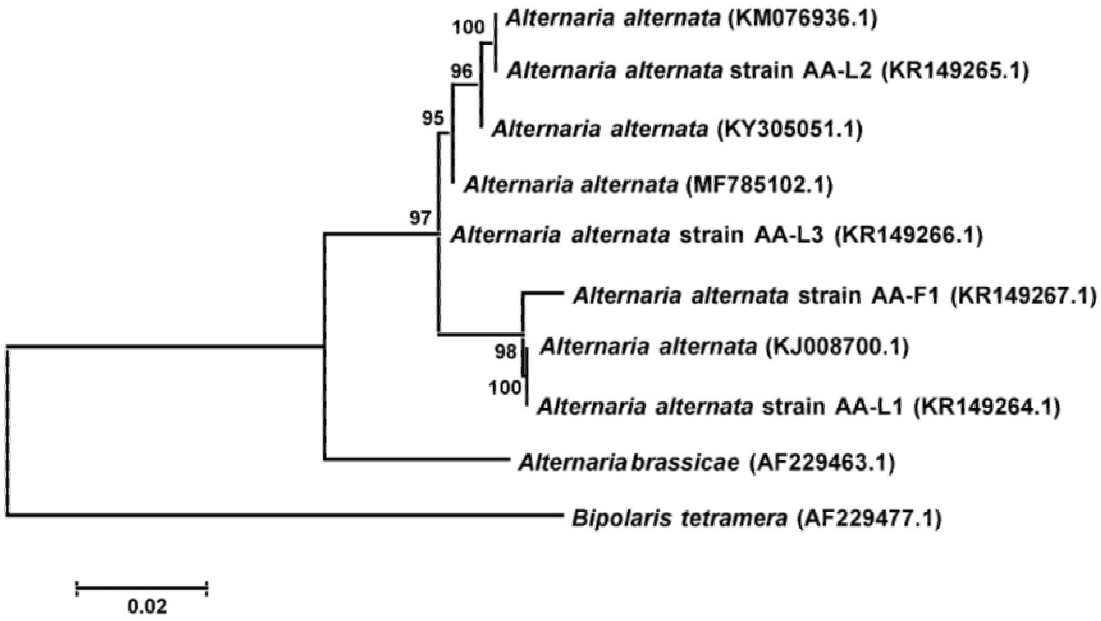

Fig. 12. Phylogenetic tree constructed with the ITS-5.8S rDNA sequences of the three strains of *Alternaria alternata* isolated from leaves of litchi (AA-L_1, AA-L_2, AA-L_3), one strain isolated from litchi fruit (AA-F_1), some other strains of *A. alternata*, and a strain of *A. brassicae* retrieved from GenBank. *Bipolaris tetramera* was used as the out-group taxon. The scale bar indicates the number of base changes per 1000 nucleotide positions in the neighbour-joining analysis.

Pathogenicity test

Three leaf blight isolates were used separately for leaf inoculation, one panicle blight isolate for panicle inoculation and one fruit blight isolate for fruit inoculation tests. Leaf inoculation tests

were conducted by spraying about 20 mL of a 10^6 conidia/mL suspension per plant onto the upper and lower surfaces of leaves of 2-yr old potted litchi plants of cv. 'Shahi'. Conidia were harvested from 6-day old PDA cultures. For each isolate, five plants were inoculated, and five control plants were sprayed with sterile water. After spraying, the foliage of plants was covered with clear plastic bags. These plants were then kept in a greenhouse at 32 ± 2 °C, with 68 ± 4 % relative humidity and a 12-h photoperiod. The greenhouse was made up of polycarbonate twin wall glazing (6 mm sheet), equipped with a proportional-integral-derivative controller for precise climate control. Panicle inoculation tests were conducted by spraying four bunches of panicles on orchard trees with 20 mL of a 10^6 conidia/mL suspension per bunch under natural daylight conditions. The panicles were then covered with thin plastic bags that had several small holes for aeration. Six developing fruit (approx. 45 days after fruit set) were inoculated with 20 mL of a conidial suspension following similar methods as for the panicle inoculation tests. For fruit decay isolate, two filter paper squares (5×5 mm) were dipped in the conidial suspensions and placed on surface sterilized fruits. Inoculated fruit were incubated in a humid chamber in the dark at 25 ± 1 °C. Ten fruit per fungal isolate were used and 10 control fruit were inoculated with sterile water. Symptom development was monitored daily. Fungi from lesions which formed on the inoculated plant tissues were re-isolated on PDA medium. The morphological and cultural characteristics of the re-isolated organisms were compared with the original isolates.

Leaf blight symptoms appeared 2 weeks after inoculation (Fig. 13), while panicle blight and fruit blight were apparent after 10 days. No symptoms were observed on control leaves, panicles, or fruit. Re-isolation of fungi with the same morphological characters was achieved from symptomatic

Fig. 13. Appearance of symptoms of leaf blight on potted seedlings of litchi after artificial inoculation of *Alternaria alternata* pathotype AA-L_3

plants/tissue, but not healthy control plant tissue, confirming the causal agent as *A. alternata*. Cross infectivity of the three leaf blight strains for panicles and fruit infection was observed with varying severity, and conversely panicle and fruit blight isolates caused leaf infection. Artificial inoculation of fruits with the isolated pathogens under *in vitro* conditions confirmed the aetiology of fruit decay. Fruit decay appeared after 3 days post-inoculation.

Disease incidence and severity

Disease incidence and severity of leaf, panicle, and fruit blights of litchi, and fruit decay were recorded during 2012-2017 in Bihar state of India. The extent of leaf blight disease developed due to natural inoculums in various nurseries was assessed. Incidence of the disease among plants (DI), percent infected leaves (IL), and percent disease severity index (PDI) were recorded. Data on disease incidence was taken in six nurseries, each having about 1000 plants. Disease incidence was calculated based on number of plants having blight symptoms on leaves using the formula:

$$\text{Disease incidence} = \frac{\text{No. of plants with blight symptoms}}{\text{Total no. of plants}} \times 100$$

Ten infected plants in each nursery were randomly observed to record percent infected leaves. Further, for estimating PDI, 30 leaves randomly selected from the same infected plants were scored individually adopting a 9-point scale (Kumar *et al.*, 2012), taking into account the percent leaf area damaged by the disease, where 1 = 0%, 2 = 1-5%, 3 = 6-10%, 4 = 11-20%, 5 = 21-30%, 6 = 31-40%, 7 = 41-60%, 8 = 61-80%, 9 = > 81% necrosed leaf area. The PDI was calculated using the formula:

$$\text{PDI} = \frac{\sum (\text{Severity grade} \times \text{No. of leaves})}{\text{Maximum grade} \times \text{Total no. of leaves scored}} \times 100$$

A total of nine orchards were surveyed during 2014-2016 at NRCL Experimental Farm and in farmers' orchard. Same orchard was surveyed every year. Panicle blight disease incidence (% infected trees in orchard), and distribution of trees in various severity categories based on percent panicles blighted on trees *viz.*, $\leq$ 20 %, 21-40%, 41-60%, 61-80% and > 80% were estimated by visual approximation. While incidence of disease was recorded on all the trees (n = 60-78) of the orchard, severity was recorded by randomly selecting ten infected trees in each orchard. Incidence of fruit blight disease was assessed in a portion of tree having about 100 fruits by counts method in four directions (N, S, E, and W) on five randomly selected trees in each orchard. These orchards were same where panicle blight data was recorded. Incidence of fruit decay (infection with visible mould growth) was recorded following random sampling at various level of supply chain and percent incidence was computed.

Leaf blight

Mean disease incidence (DI) among plants in various nurseries from July 2012 to December 2013 varied from 23.2 to 82.6 %, highest during December and April. The mean and range of disease incidence among leaves (IL) on infected plants were 48.4-64.17 % and 41.0-73.2 %, respectively. The mean percent disease severity index (PDI) varied from 49.8 to 71.2. During November 2016 to March 2017, mean DI among plants in different nurseries was between 7.1 to 14.6 %, IL on infected plants was 19.5-35.2 and PDI was 43.3-57.3. Thus, it is apparent that a substantial number of leaves were blighted due to the disease, hampering plant growth in nurseries. Initially senescing leaves were infected but with the progression of the disease all the leaves become blighted except a few upper leaves.

Panicle blight

On cv. 'Shahi', mean incidence of panicle blight at NRCL experimental farm was 63.3 % (ranged 43.1-77.1 %) in 2014, 38.3 % (ranged 28.5-50.0 %) in 2015 and 17.3 % (ranged 6.3-27.8 %) in 2016. Mean disease incidence gradually decreased from 63.3 % in 2014 to 38.3 % in 2015 and 17.3 % in 2016. The data on mean distribution of trees in different levels of panicle blights on cv. 'Shahi' showed that trees in $\leq$ 20 %, 21-40 %, 41-60 %, 61-80 % and >80 % blighted panicles in surveyed orchards were 59.0-70.0 %, 6.2-24.8 %, 5.4-14.8 %, 3.1 %-7.9 % and 1.1-3.6 %, respectively. This indicated that the maximum numbers of trees were having less than 20 % blighted panicles. Mean disease incidence of panicle blight (tree basis) on cv. 'China' in farmers orchard was 34.4 % (ranged 23.8-47.6 %) in 2015 and 35.9 % (ranged 17.0-58.9 %) in 2016. Similar to cv. 'Shahi', greater number of trees of cv. 'China' in orchards were observed to have less than 20 % blighted panicles. The major loss to the crop, in terms of quantum of fruits damaged, occurred due to infection of panicles leading to partial blighting of panicles on a portion of tree. Though most of the trees in surveyed orchards had diseased panicles below 20 %, it resulted in heavy loss in terms of reduction in potential fruit harvest.

Fruit blight

The mean disease incidence of fruit blight on trees in farmers' orchard in Muzaffarpur, Bihar during June 2014 varied from 7.7-10.2 % while the range was 2.9-12.9 %. Similar trend of fruit blight incidence on trees in orchards were observed during 2015 and 2016 seasons where the mean incidence remained between 6.6 to 17.3 %. The distribution of blighted fruits in the tree in four cardinal directions (N, S, E, and W) showed no association with particular direction. The fruit blight incidence was sporadic in nature.

Fruit rot/decay

Fruit decay is one of the major obstacles in the postharvest fruit chain reducing the commercial value of litchi fruit. The maximum fruit decay or rotting was observed at retailer level in the supply chain. The study revealed that fruit rot/decay was caused by four species of fungi: *Alternaria alternata, Colletotrichum gloeosporioides, Aspergillus niger* and *A. flavus*. Among these, *A. alternata* was the dominant species (86.7 %) followed by *C. gloeosporioides* (7.2 %), *A. flavus* (3.9 %) and *A. niger* (2.2 %) during 2012. Similar observations were recorded during 2013 (Kumar et al. 2016a). Artificial inoculation of fruits with the isolated pathogens under *in vitro* conditions confirmed the aetiology of fruit decay of litchi. Micro-cracks during fruit development and postharvest handling can provide a port of entry for decay pathogens that colonize the fruit surface. In India, postharvest diseases caused by *Aspergillus, Cylindrocarpon, Botryodiplodia* and *Colletotrichum* were reported by Prasad and Bilgrami (1974). Liu et al. (2006) reported that in Guangdong and Hainan (China), *C. gloeosporioides* was the main pathogen causing postharvest decay which came mainly from fruits with latent infection prior to harvest. The predominant fungal genera associated with litchi in South Africa were *Phomopsis*, *Pestalotiopsis*, *Penicillium*, *Trichoderma*, *Alternaria*, *Botryosphaeria* and *Fusarium* spp. (DeJager et al. 2003). Many *Penicillium* spp. have been isolated pre-and post-harvestly from litchi, of which *P. expansum* was reported as the major pathogenic species (Jacobs and Korsten 2004). *A. alternata* was reported as the most common pathogen associated with fruit decay of litchi in India by Kumar et al. (2016 a).

It is clear from the data that the incidence and severity of disease at different growth phases were considerably high to cause economic loss to the crop, and if a cumulative loss at different stages is considered, the pathogen *A. alternata* becomes much more important. The cv. 'Shahi' is the leading cultivar in Bihar and adjoining states of India followed by cv. 'China'. While cv. 'Shahi' matures around 20th May, cv. China matures in the 1st week of June. These two cultivars did not show any differential susceptibility to the disease at different phases of crop.

Ecophysiology of *Alternaria alternata*

Studies were conducted to assess the effect of different medium, carbon source, pH, temperature, relative humidity and exposure to UV radiation on growth and sporulation of *A. alternata* under *in vitro* conditions. Results showed that the maximum mycelia growth rate was on Host Leaf Extract Dextrose Agar but sporulation was the maximum on Potato Dextrose Agar medium. The mycelia growth was more with dextrose as carbon source but the maximum dry weight was recorded in maltose amended medium. Optimum pH for mycelia growth was 6.0, and below or above this pH, mycelia growth was slow. It was evident that the maximum mycelia growth rate and germination of conidia were at 30 °C. The thermal death point of *A. alternata* was found to be 45 °C with 10 min. exposure. The maximum mean growth rate and germination percent was

recorded at 100 % relative humidity. The growth rate decreased with increasing exposure time to UV-B rays up to 20 min, but at exposure time 25 and 30 minutes, colony changed to profuse mycelia growth with smaller sporulating zone in the centre compared to untreated dishes. The findings of the study will be helpful in understanding epidemiology and devising management strategies of this disease.

The study generated important information about how the environment, both physical and biological, interacts with the growth and sporulation of *A. alternata*. The study on media revealed that host leaf extract favoured mycelial growth of this necrotrophic pathogen. Among the carbon source, dextrose was best for mycelia growth and maltose for higher biomass. *A. alternata* preferred an acidic pH (6.0) for mycelia growth. The pathogen grow well between 30-35°C though it tolerated a temperature up to 50 °C as conidia could germinate at these temperatures. From flowering to fruit ripening, a temperature regime of 28-40 °C prevails during day in litchi growing areas, the mean maximum temperature (Tmax) being 32-35 °C. This finding corroborates with the field observation of disease incidence and severity of panicle and fruit blight caused by the pathogen. Further, findings indicated that growth and conidial germination was fairly good to the best between 60-100% RH, 100% RH being most congenial. In field conditions, though during daytime prevailing humidity is lower (60 to 85%), the dew drops on leaves help conidia to germinate and cause new infections in early hours of the day. This observation corroborates with the disease incidence and severity of panicle and fruit blight in orchards. The study demonstrated tolerance of pathogen to UV-radiation. The airborne conidia of *A. alternata* thus can avoid the damage from UV-radiation and cause new infection enabling its spread and survival under prevailing harsh weather of summer. Thus, this study is not only helpful to understand growth and sporulation conditions of the pathogen but also corroborate with epidemiology of panicle and fruit blight disease in orchards.

Epidemiology

Survival and spread of pathogen

Infected fallen leaf litters were collected from litchi orchards and stored at two conditions *viz.*, in a BOD incubator (27 ± 1 ºC) and at ambient conditions (room temperature) for 12 months. Observations on survival and viability of *A. alternata* propagules were recorded at monthly interval by plating infected bits onto PDA, and by observing the temporary mounts prepared from infected bits under brightfield microscope. Results showed that *A. alternata* propagules (conidia) were viable in infected leaves up to 7 month when stored in BOD incubator while at ambient conditions it remained viable only up to 5 month (Fig. 14). Another set of monitoring experiment revealed that in orchards, the pathogen survived throughout the year on infected leaves in lower canopy of trees and also on nursery plants. These acted as primary source of inoculums for the infection of panicles and fruits of litchi during fruiting season.

Fig. 14. *Alternaria alternata* in tissue of a fallen leaf under 20 × objective of brightfield microscope

Spatial and temporal variation in number of air-borne conidia

Spatial and temporal conidial population of *A. alternata* in vicinity of tree canopy in orchards, and in nurseries was monitored by air sampling. Petri dishes containing PDA amended with 0.5 mg/mL of streptomycin sulphate were exposed for 5 minute at different heights (Fig. 15). For trees in orchard, plates were exposed at 3 ft, 6 ft and 12 ft from soil surface while inside nursery plants at 1.5 ft and 3.0 ft above ground level. Sampling time was morning 6 AM, after noon 12 PM and evening 6 PM. After exposure of the plates, lids were returned, sealed with parafilm, brought to laboratory and incubated at 27 ± 1 °C for five days. Colonies which developed in plates were examined under a stereoscopic microscope to identify colony of *A. alternata* among them (Fig. 16). Results showed that the maximum numbers of conidia were present below tree canopy at 3-6 feet height. Temporal variation showed that the maximum conidia in air were present between 6.00-10.00 AM in morning hours.

Weather parameters vis-à-vis disease

Weather parameters throughout flowering and fruiting period of litchi during 2014-2016 were: T_{max} = 31.1-40.7 °C, Tmin = 17.4-27.2 °C, RH_{max} = 60.0-85.0%, RH_{min} = 23-57%. The analysis of prevailing weather conditions revealed that a temperature of about 28-30°C and humidity 60 to 85% were congenial for panicle and fruit blight disease. Trendlines plotted on weather graph showed that the disease severity was more between Tmin 20-22 °C and Tmax 32-35 °C. This observation corroborates with Maheshwari et al. (2000) who reported an excellent sporulation in

Fig. 15. Sampling to trap air borne conidia of *Alternaria alternata* in vicinity of litchi tree canopy; Top - At 3 ft height, Middle - At 6 ft height, Bottom - At 12 ft height

A. alternata under *in vitro* condition at 25-30 °C with growth optima at 28 °C. An increase in infection, lesion development and expansion of *A. alternata* on *Paulownia* trees from 15-25 °C and decline between 30 to 37 °C was observed by Pleysier et al. (2006) in Western Australia. A close perusal of the data on severity of disease vis-à-vis prevailing weather indicated that for panicle and fruit blights, a dry weather was not a constraint for infection however, rains favoured rapid development of the disease. These results agree with those of Timmer et al. (2003) who observed that when the conidium of *A. alternata* landed on a leaf, it waited until the night-time dew, and then germinated. A negative correlation between humidity and disease severity (r = -0.55) of leaf blight of pea caused by *A. alternata* was also reported by Gahalain et al. (1999). Temperature influenced infection, lesion development and expansion caused by *A. alternata*. The dispersal of the conidia took place by air. Conidia could be propelled into air by a shift from wetness to dryness, a rapid increase in humidity or exposure to the red light (Rotem 1994). *A. alternata* is a hardy fungus and can live in extreme conditions. Due to comparatively big sized spores and presence of the dark pigment melanin, *A. alternata* can float in the air and avoid the damage from UV-radiation (Abbo 2012). Since, litchi is cultivated over a large area in Bihar and adjoining states of India, the pathogen has the potential to spread and cause heavy loss to crop.

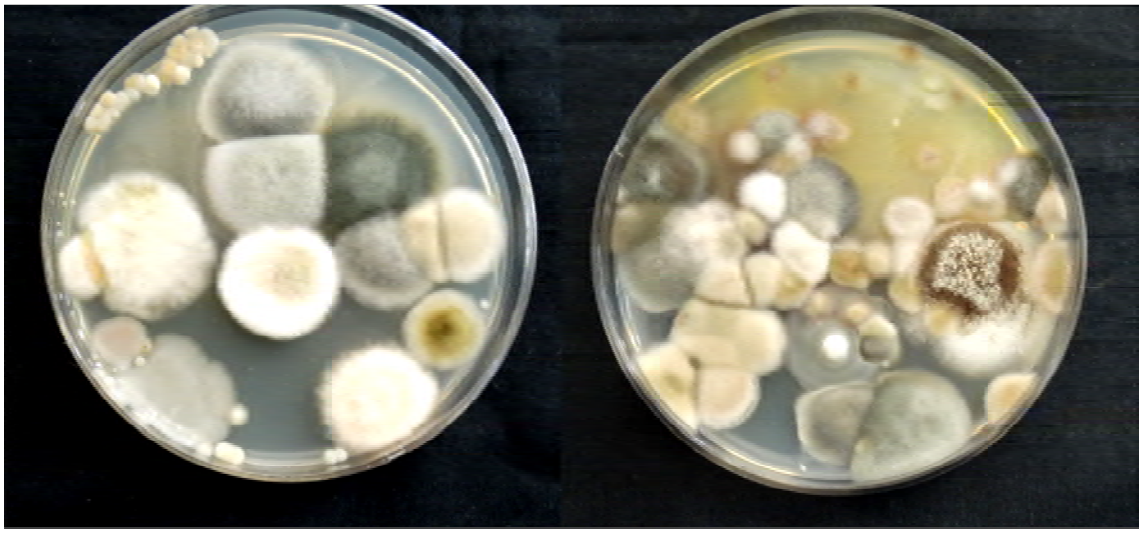

Fig. 16. Colony developed by airborne conidia of *Alternaria alternata* trapped on PDA in Petri plates

Disease management

Antifungal activity of some ethnomedicinal plant infusions

Biopesticidal properties of some ethnomedicinal plant infusions were explored as alternative management strategy for *Alternaria* disease of litchi. Ethnomedicinal plants such as Catnip (*Nepeta*

cataria; Lamiaceae), Guma (*Leucas aspera;* Lamiaceae), Indian Heliotrope (*Heliotropium indicum;* Boraginaceae), Garden spurge or Baridudhi *(Euphorbia hirta;* Euphorbiaceae), Aak (*Calotropis procera;* Apocynaceae), Datura (*Datura stramonium;* Solanaceae), Karanj (*Pongamia pinnata;* Fabaceae), Bhainth or bhant (*Clerodendrum viscosum*; Lamiaceae), Congress grass (*Parthenium hysterophorus;* Asteraceae), Garlic (*Allium sativum;* Amaryllidaceae), Neem (*Azadirachta indica;* Meliaceae) and turmeric (*Curcuma longa;* Zingiberaceae) were used in the study. Aqueous and methanolic extracts of these plant infusions, and materials used in 'Zero budget natural farming' were evaluated for their antifungal activity

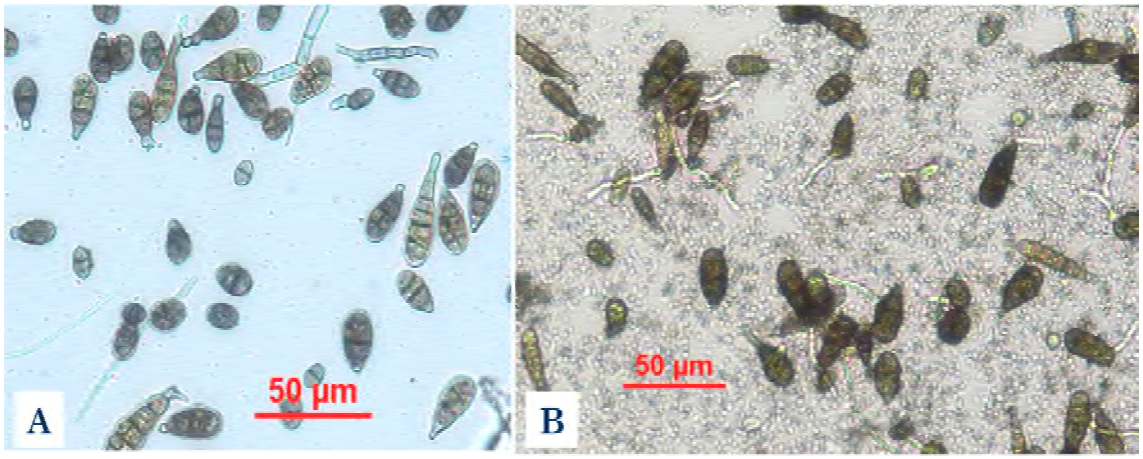

Fig. 17. *In-vitro* spore germination test (Slide Germination Method):
A. *Calotropis procera extract*, B. Control

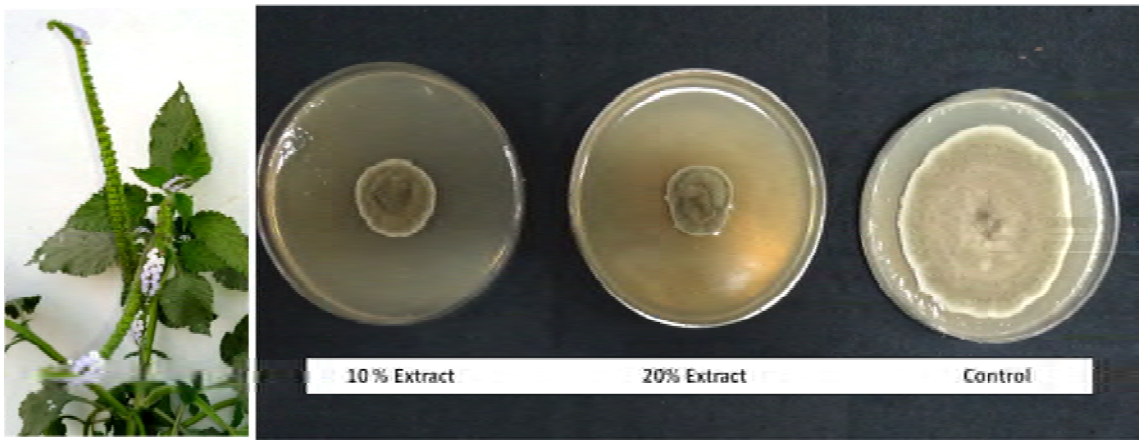

Fig. 18: Left- An Indian Heliotrope (*Heliotropium indicum)* plant, Right- Fungistatic effect of 10 % and 20% aqueous extract of Indian Heliotrope plants on colony growth of *Alternaria alternata* compared to control (Distilled water)

against *Alternaria alternata* under *in-vitro* conditions. The preliminary results suggest that the leaf extracts of *Datura stramonium*, *Calotropis procera* and *Heliotropium indicum* possess antifungal compounds (Figs. 17, 18) that can be harnessed for development of formulations or simply as a crude extract application for the management of *A. alternata*. Further experiments may focus on finding the bioactive constituents present in these plant infusions and feasibility of their application under field conditions.

Evaluation of fungicides for management of Alternaria disease

a) Greenhouse conditions

Twelve different fungicides (copper oxychloride, mancozeb, thiophanate methyl, carbendazim, difenoconazole, hexaconazole, propiconazole, propioneb, chlorothalonil, azoxystrobin, metiram + pyraclostrobin, and mancozeb + carbendazim) were evaluated under greenhouse conditions during 2015-2018 for management of leaf blight disease in nursery plants. Among plants of each treatment one infector plant was kept. To ensure sufficient disease pressure, spray application of conidial inoculum of *A. alternata* (1×10^6 conidia/ mL) was also done on plants. Results showed that among different treatments, thiophanate methyl (0.14 %) or difenoconazole (0.025 %) or azoxystrobin (0.023 %) were very effective in controlling leaf blight disease, PDI being below 10.0 % as against up to 89.6 % in control plants.

b) Field conditions

The same set of treatments as in greenhouse conditions was tried against panicle and fruit blight disease under natural infection field conditions during 2015-2018. Efficacy of these fungicides in controlling panicle and fruit blight disease were similar to efficacy against leaf blight disease observed on nursery plants. Mean incidence of panicle blight was below 3.0 % in the effective fungicidal treatment as against up to 27.0 % in control trees over the years. The disease incidence of fruit blight was 1.7-3.6 % in the effective fungicidal treatment as against up to 14.7 % in control trees over the years.

Integrated management of Alternaria diseases of litchi

- Follow orchard sanitation by removing and destroying infected leaves.
- Augment natural plant defense by soil application of microbes such as Trichoderma, Mycorrhiza etc along with compost/farm yard manure.
- Spray thiophanate methyl (0.14 %) or difenoconazole (0.025 %) or azoxystrobin (0.023 %) or carbendazim (0.1 %) on appearance of first visual symptoms of disease.
- For panicle and fruit blight phase, one spray just after panicle emergence but before flower opening, and another spray 20 days before harvest (colour-break stage) of fruits.

- Pre-harvest spray of fungicides at colour-break stage help to prevent anthracnose and extend post-harvest life of fruits
- Harvest fruits during early morning hour (4.00-8.00 AM) and avoid any mechanical injury to fruits.
- Prompt pre-cooling (temperature 4 °C, RH 85-90 % and maintenance of optimum temperature and relative humidity during transportation of fruits helps to prevent fruit rot.
- Fruits may be treated by sulphur fumes if allowed by importing countries. For this, fruits are placed in a closed chamber where 50-100 g sulphur per m^3 of air space is burnt for 20-30 minute.
- For transportation, use corrugated fibre board (CFB) boxes of 2 kg capacity properly unitized for stacking. Other measures include biological control of decay pathogens and use of a 10-15 % CO_2-enriched atmosphere for packaging.

Conclusions

Diseases are one of the constraints to the production of litchi fruit. They indirectly reduce yield by debilitating trees, and directly reduce the yield or quality of fruit before and after they are harvested. Since 2014, *A. alternata* has been an important pathogen of litchi in India causing blights of leaf, panicle, and fruits. The cultivation of litchi over a large area in Bihar and adjoining states of India provides the pathogen with the opportunity to spread and cause heavy crop loss. A post-harvest loss of 35-44 % of litchi fruits has been reported by Kumar et al. (2016a), and much of this is due to decay caused by *A. alternata*. Thus, considering cumulative loss incurred at different stages of the crop, this pathogen has become a serious problem on litchi. Monitoring is an important tool to keep tracks of the pathogens and their potential damage. Further research is warranted to investigate the epidemiology and develop strategies for management of this economically important disease.

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About ICAR-NRCL

The ICAR-National Research Centre on Litchi is premier national institute for conducting research and developments on litchi and provides leadership at national level. It also acts as a national repository of information on litchi production, processing, value addition, and provides consultancy services to end users. The ICAR- NRCL was established on 6th June, 2001 by Ministry of Agriculture, Government of India under the aegis of Indian Council of Agricultural Research. The Centre is located at Mushahari, on Muzaffarpur-Pusa Road at 26°5'87" N latitude, 85°26'64" E longitude at an elevation of 210 m. The area has typically subtropical climate with an average annual rainfall of 1100-1300 mm. Soil conditions of the area are alluvial with sandy loam texture and are calcareous. The research farm of the centre is spread over an area of 40 ha. Mandate of the Centre are - applied and strategic research on genetic resources and production technologies for enhanced, sustained and safe production of litchi, and transfer of technology and capacity building of stakeholders for enhancing and sustaining productivity of litchi.

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