Occurrence, severity and association of fungal pathogen, *Botrydiplodia theobromae* with sudden death or decline of tree bean (*Parkia timoriana*, (DC.) Merr) in North Eastern India

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Tree bean, *Parkia timoriana* is one of the most important perennial tree legume vegetable crops in north eastern region (NER) of India. Recently, sudden death or decline is emerging as a major constraint in the production of tree bean. The prevalence, intensity and etiology of this disease have not been extensively studied. The present work was aimed at studying the symptomology, severity and etiology of sudden death or decline of tree bean in the north eastern region of India. Typical symptoms of tree bean decline were initiated with wilting of half portion of the tree, excessive shedding of leaves, gradual drying up leading to death, and secondary infestation by insects on dead trees. The highest disease severity of 68.5% was observed in warmer regions like Kolasib, Mizoram. During survey, small bark borer was recorded in all districts and levels of infestation rate (holes/ft^2 of log) reached maximum up to 25.49 in Hnanthial of Lunglei district. Young trees in the range 1–10 years with 57.32% (917 trees) of total trees (1600) infected were most susceptible to the disease compared to 12.58% (201 trees) infection in old trees in the range 21–30 years. The frequently associated pathogen with sudden death or decline of tree bean was identified as *Botrydiplodia theobromae* at the Indian Type Culture Collection, ICAR-IARI, New Delhi. It was the most aggressive species and proven to be pathogenic to tree bean following artificial inoculation.

Keywords: *Botrydiplodia theobromae*, north eastern India, *Parkia timoriana*, sudden death or decline disease, tree bean.

TREE bean (*Parkia timoriana*, (DC.) Merr.) syn. (*Parkia roxburghii*, *Parkia javanica*) is the most popular nutritious legume vegetable tree growing luxuriantly throughout the north eastern region (NER) of India and south east Asian countries. NER is regarded as the primary and secondary source of origin of *P. roxburghii*[1]. The tree bean is distributed in regions ranging from humid tropical valley to subtropical climate with altitude ranging from 400 to 1200 m amsl and can grow to up to a height of 30–35 m. It is extensively grown in the homestead, *Jhum*, road sides and forests of Manipur, Meghalaya, Mizoram, Nagaland and some pockets of North Cachar Hills and Sibsagar of Assam[2]. Tree bean plays a key role in socio-economic, environmental[3,4] and nutritional security in the region[3,5–7]. It can be consumed in all developmental stages starting from the green tender, full bloom of flowers, pods to matured seeds[8]. Mass scale decline or sudden death of tree bean (*Parkia* spp.) trees was reported from Manipur, India during 2002 and the cause was linked to an insect stem borer, *Bactocera* sp.[9]. Later on, synchronous decline/sudden death of tree bean trees was reported from various parts of the valley and hilly regions (*Jhum*) of Manipur, Nagaland, Mizoram and Meghalaya, which seriously affected the socio-economic situation of the growers[10,11]. The disease significantly resulted in the decline in production of pods and led to import of huge quantities from neighbouring countries mainly, Myanmar and Bangladesh[12]. The symptoms of this disease began as drying up of half the tree, falling of leaflets, wilting, excessive gummosis, easy to peel off bark, and later the whole tree dried up. In the early stage, longitudinal blackening was observed in the cross-section of the infected tree trunk. Tiny holes made by an unknown bark borer were frequently seen on the diseased tree wood which was suspected to act as a vector. The myth behind the sudden death of tree bean trees is that, it is due to the emission of radiation from mobile towers, absence of bat pollinator, climate change, insect–pest infestation and

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pathogen complex infection[2,13,14]. Seedling wilt of tree bean caused by *Verticilium dahliae* was reported by Rajesh *et al.*[15], during 2012 from the State department forest nursery of Chandel, Manipur. During 2003, a pod borer *Cadra cautella* isolated from the pods of tree bean collected from fields and stored, have been well documented[10]. There were no systematic surveys conducted to assess the role of pathogenic fungi on declining tree bean within North Eastern areas. However association of multiple fungal pathogens with the decline diseases of other tree species has been well documented[16,17]. It has been reported that the cause of sudden death of mango in Pakistan is due to the establishment of vector–pathogen relationship between *Lasiodiplodia theobromae* and bark borer *Hypocryphalus mangiferae*[16]. Similar is the confirmed vector–pathogen relationship between elm barks borers (*Scolytus scolytus*, *S. multistriatus*) and (*Ophiostoma ulmi*, *O. neo-ulmi*) in oak[18]. *Raffaelea quercus-mongolicae* causing oak wilt mortality in South Korea, vectored by wood-boring ambrosia borer, *Platypus koryoensis* was well established[19,20]. *Raffaelea lauricola* causing avocado laurel wilt and its vector bark borers (*Xyleborus glabratus*) evolved to colonize the xylem tissue of woody plants as they have symbiotic relationship[21]. The role of most frequently found bark borer on diseased tree bean as a vector is yet to be identified. The bark borers prefer diseased wood and the saw dust comes out of tiny holes made by them describing about this pattern. The socio-economic and ecological importance of tree bean in these regions warranted such an investigation on the potential biotic (pathogens and insect pests) and abiotic stress. Currently, no research data is available regarding the nature of decline, occurrence, severity and known behaviour of pathogens recovered from diseased tree beans from NER of India. Keeping in view the status of sudden death or decline of tree bean trees and the magnitude of loss due to this disease, this study was undertaken to characterize the symptomatology of tree bean decline, evaluate the severity and prevalence of the disease, identify the aetiological agent, and evaluate pathogenicity and aggressiveness of the pathogen.

Materials and methods

Field survey, sampling sites and specimen collection

An extensive random survey was carried out during 2014–15 in the tree bean growing areas, viz. *Jhums*, homestead and road sides of Mizoram. In each district, four villages and 50 trees of different age groups were randomly selected. A total of 1600 tree bean plants were examined in 32 villages covering all eight districts of Mizoram. The geographical details of villages surveyed were recorded with (Global Positioning Services (GPS), Model: GPSMAP 76CSx, Garmin, USA) data (Table 1 and Figure 1). Information on the number of dead trees in surveyed areas was obtained from growers. The trees were examined visually as well as chopped to know the presence of decline signs and symptoms such as excessive gummosis, wilting branches, excessive leaf shedding, rotting, trash, and black streak on diseased tree. Trees infested by bark borer were identified by the presence of tiny holes with the exudation of creamy white powdery saw dust from the bark. Trunk tissue of one asymptomatic tree per village was also collected. Small logs (5–6 cm long and 4–5 cm in diameter) were taken from symptomatic trees (one per plant) and healthy plant. Bark borer adults were caught from the tunnel of infected trunk of tree beans by peeling off the bark from the collar or above the ground stem portion.

Disease symptomatology, severity and age wise susceptibility level

The type of symptoms produced on tree bean due to sudden death or decline were from the fields surveyed. The symptoms that included drying up of half of the main branches, excessive gummosis, bark splitting, blackening of vascular bundles, excessive tiny insect holes with emission of trash and leaf shedding were examined to determine the disease severity and intensity. Sudden death or decline of tree beans was rated by using a 0–7 scale, where scale 0 was the highly resistant with no disease severity. Scale 1, 2, 3, 4, 5 and 6 consisted of 1–10%, 11–20%, 21–30%, 31–40%, 41–50% and 51–60% disease severity respectively, and scale 7 was the highly susceptible case with >60% intensity[17]. Disease severity index (DSI) was calculated by using the formula[22]

$$\mathrm{DSI\%} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times 7} \times 100.$$

Assuming the possible variation in age response of tree bean to sudden death or decline, four ranges of age, viz. 1–10, 11–20, 21–30 and >31 years based on grower's feedbacks were grouped to determine the age-wise susceptibility level.

Fungal isolation and identification

Diseased samples collected from symptomatic plants from each district of Mizoram were washed under clean tap water and dried. Diseased tree parts like tap roots, crown, middle and top portion of tree, asymptomatic healthy part, borer, gum and trash were isolated. Small pieces of wood (5 mm^3), insect, gum and trash were submerged in 2.5% sodium hypochlorite for 2 min and washed thrice in sterile distilled water. Samples were

Table 1. Geographical coordinates of the survey points of different villages of Mizoram

District	Villages	Sites ID	Geographical coordinates of villages		
			Altitudes (m)	Latitudes	Longitudes
Kolasib	Kawnpui	Kp	820.10	24°2′35.99″N	92°40′19.24″E
	Kolasib (Project Veng)	Kb	661.72	24°13′28.43″N	92°40′33.69″E
	Bilkhwalthlir	Bt	457.81	24°11′7.26″N	92°32′13.66″E
	Buchangphai	Bp	184.40	24°24′25.09″N	92°41′13.94″E
Aizawl	Tuirial zau	Tz	184.40	24°21′23.41″N	92°53′9.69″E
	Keifang	Kf	1082.95	23°40′16.15″N	92°57′40.71″E
	Shiphir	Sp	1036.32	23°45′20.12″N	92°43′48.49″E
	Tlungvel	Tv	1150.90	23°36′17.2″N	92°51′14.29″E
Champhai	Khawzawl	Kz	1236.88	23°31′59.24″N	93°11′5.66″E
	New Champhai	Nc	1547.77	23°28′18.01″N	93°20′9.24″E
	Zotlang	Zt	1480.11	23°27′17.15″N	93°20′39.51″E
	Vapar	Vp	1416.15	23°37′9.67″N	93°20′58.27″E
Serchhip	Chekawn	Ck	867.16	23°11′9.89″N	93°1′5.51″E
	Kietum	Kt	1002.79	23°14′16.27″N	92°54′33.88″E
	Thenzawl	Tz	750.72	23°16′43.04″N	92°46′19.75″E
	East Lungdar	El	1357.88	23°12′22.72″N	93°4′55.21″E
Lunglie	Hnanthial	Ht	769.62	22°57′52.23″N	92°55′36.96″E
	Tuipui-D	Td	275.84	22°54′12.5″N	92°55′56.96″E
	Vanhne	Vn	865.02	22°55′9.55″N	92°42′45.64″E
	Tawipui S&N	TSN	940.61	22°41′30.72″N	92°50′25.73″E
Saiha	Meisavaih	Mv	995.78	22°29′29.59″N	92°58′33.18″E
	Maubawk	Mb	845.82	22°25′42.16″N	92°58′16.85″E
	Kalchaw-E	Ke	181.13	22°23′50.04″N	92°57′28.5″E
	Lungban	Lb	1040.89	22°28′16.4″N	93°6′18.15″E
Lawngtlai	Rawlbuk	Rb	1183.84	22°40′24.12″N	92°59′51.41″E
	Sihtlangpui	Sp	415.75	22°25′3.78″N	92°56′13.35″E
	Chawnhu	Cn	1060.70	22° 30′10.21″N	92°53′37.71″E
	Diltlang	Dt	407.82	22°29′33.48″N	92°43′32.64″E
Mamit	Dampui	Dp	1038.73	23°49′31.07″N	92°29′13.69″E
	Marpara S	Ms	77.74	23°16′4.81″N	92°24′23.72″E
	West Phaileng	Wp	740.70	23°41′10.44″N	92°29′16.39″E
	Lengpui	Lp	418.93	23°50′22.98″N	92°38′3.34″E

plated on potato dextrose agar medium (PDA, Himedia, India) amended with streptomycin (30 ppm) and incubated at 28°C for 7 days[16]. Fungal isolates developed from diseased tissues were transferred to fresh PDA plates and pure cultures were obtained. Morphological characteristics (mycelium colour, conidial size, shape, colour, striation, septation, conidiogenous cells and presence of paraphyses) of the isolates were observed under a microscope. Representative cultures were deposited at the Indian Type Culture Collection (ITCC), Division of Plant Pathology, ICAR-IARI (New Delhi, India) for identification.

Pathogenicity test

Pathogenicity tests with three fungal species (*Botrydopiplodia theobromae*, *Pestalotiopsis guepini* and *Pestalotiopsis disseminata*) isolated from the tree bean samples were performed to satisfy Koch's postulates. Subsequently, the symptom severities associated with each pathogenic fungus were evaluated (as a measure of relative species virulence and aggressiveness). One-week-old cultures of fungal isolates, *B. theobromae*, *P. guepini* and *P. disseminata* grown on PDA at 28°C were used for the test. The isolates were inoculated to the potted one-year-old healthy tree bean plants by removing a piece of stem with a sterilized blade from an actively growing green shoot and placing a colonized agar disc (5 mm) on the cut portion, which was then covered with sterilized cotton and sealed with parafilm (Figure 7). A total of thirty healthy seedlings were inoculated with two isolates of each pathogen. Finally, sterile distilled water was injected with a dropper into the inoculated area to moisten the cotton. Five healthy seedlings were inoculated with pieces of non-colonized PDA. Five replications for each treatment were made under completely randomized design (CRD). The plants were maintained in a non-controlled greenhouse at room temperature (about 26°C) and kept under observation for 7 months. The virulence (aggressiveness) of the isolates was determined by evaluating the number of oozings (gummosis), size of the necrotic lesions produced, days taken for shedding of leaf and wilting. Test pathogen from wilted seedlings was re-isolated for confirmation of Koch's postulates. District-wise data regarding disease severity percentage of all surveyed locations, and susceptibility age response of tree bean to decline from these different regions were subjected to statistical analysis using the analyses of variance (ANOVA). Treatment means were compared by the New Duncan's Multiple Range Test (DMRT) at ($P \leq 0.05$) through statistical analysis system (SAS, 2016).

Results and discussion

Survey, disease severity, symptomatology and susceptibility level

The survey which was conducted during *Kharif* seasons of 2014 and 2015 in eight different districts, i.e. Kolasib, Aizawl, Mamit, Saiha, Champhai, Lunglei, Lawngtlai and Serchhip of Mizoram revealed that the severity of sudden death or decline of tree bean varied from 11.77% to 76.30%. The trees which were grown in *jhums*, road sides and homesteads found to be infected and showed signs of sudden death or decline in all the surveyed areas of Mizoram (Table 1). Among the villages, Kawnpui area in Kolasib district recorded the highest severity of sudden death or decline with 76.30%; the least severity of 11.77% was recorded from Mamit area of Mamit district. The next highest severity level was recorded in most places like Kolasib (Project Veng) (66.50%), Bilkhwalthlir (62.26%) and Buchangphai (58.94%) of Kolasib district; Lungban (66.47%), Kalchaw-E (57.51) and Maubawk (55.61%) of Saiha district; Zotlang (55.62%) of Champhai district and Tawipui S&N (57.86%) of Lunglei district.

The bark borer infestation rate was recorded in the range of 1.57–26.12 holes/ft^2 of log (Table 2). Maximum borer infestation rate (holes/ft^2) was recorded from Tuipui D (30.44), followed by Vanhne (26.12), Hnanthial (25.49), Meisavaih (21.86), Buchangphai (21.60) and Kawnpui (20.56). The bark borer infestation rate (holes/ft^2) was least in Bunghmun (1.57) and Thenzawl (3.04) of Serchhip district and Marpara (4.52) and Lengpui (5.48) of Mamit district. In district-wise studies (Figure 3), the disease severity appeared comparatively higher at Kolasib (66.00%), Saiha (56.27%) and Champhai (52.76%) districts. Bark borers were found predominantly in all the districts with maximum infestation recorded up to 24.27 hole/ft^2. The least bark borer infestation was recorded from Serchhip district with 3.66 hole/ft^2. Similar findings were also reported from other tree species like mango and pear, which were badly affected by *Botryodiplodia theobromae* causing dieback and bark canker disease in India[23,24].

Symptomology of sudden death or decline of tree bean was carried out from initiation of disease till death. The typical sign and symptoms of the disease comprised wilting, drying up and shedding of pinnate leaflets, drying up of branches, excessive gummosis, infestation by bark borer, presence of holes and emission of trashes (Figure 4 *e*–*g*). Freshly dead trees were closely examined by dissecting each plant part, i.e. tap root, collar region, middle part of trunk and end point of the tree. The diseased tree's trunk always showed signs of vascular black streaks running longitudinally and excessive gummosis. During initial infection, dropping of leaflets and wilting and later excessive shedding of leaflets due to blocking of nutrients or lack of water were observed, gradually leading to death of the tree. The diseased tree typically showed two types of symptoms. First, half of the tree started drying up and gradually the tree health and vigour is lost, ultimately leading to decline within one year. Secondly, the tree started wilting and shedding leaflets. The whole tree started drying up instantly (Figure 4 *a* and *b*) leading to death within one–two months[16].

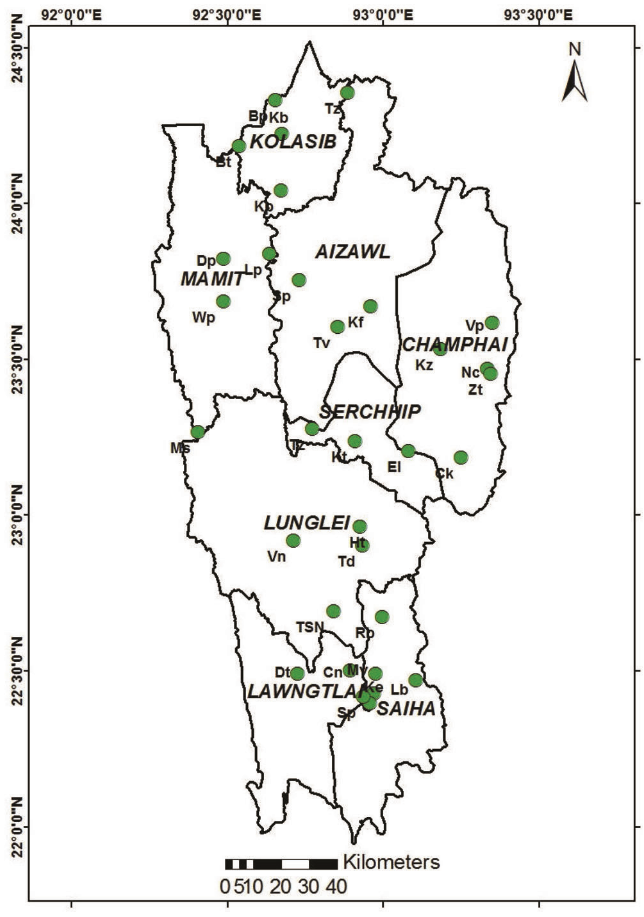
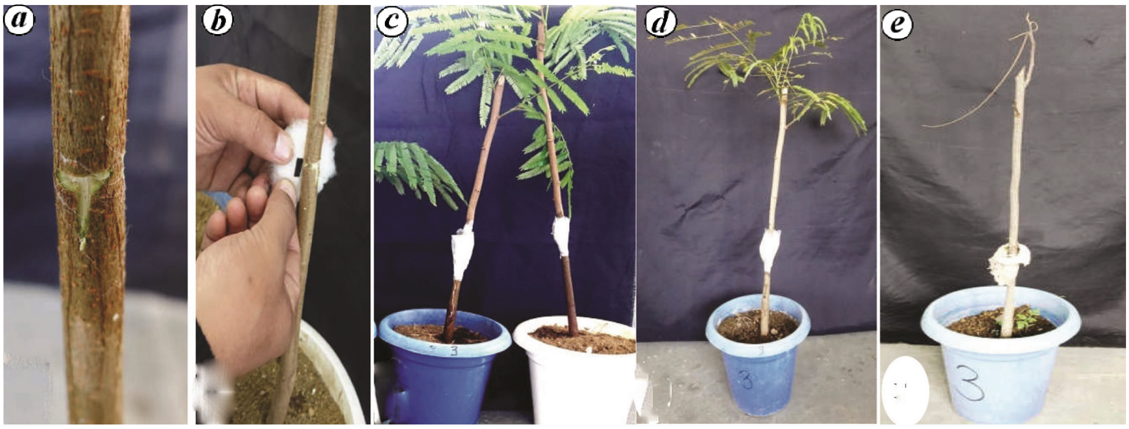


Figure 1. Map showing the districts and villages surveyed for tree bean (*Parkia timoriana*) areas in Mizoram.



Figire 2. Result of pathogenicity test of frequently isolated pathogen, *Botrydiplodia theobromae* on young seedlings of tree bean. ***a***, T-shaped girdled; ***b***, inoculation of mycelium of *B. theobromae*; ***c***, parafilm wrapped inoculated seedlings with *B. theobromae*; ***d***, wilting symptom development after two months of inoculation; ***e***, complete wilt and death of tree bean seedlings after seven months of inoculation of pathogen, *B. theobromae.*

Table 2. Status of tree bean sudden death/decline disease incidence (%) and bark borer infestation rate (hole/ft^2) in different districts of Mizoram

Districts	Villages	Disease incidence (%) ± SE	Bark borer* infestation rate (hole/ft^2) ± SE
Kolasib	Kawnpui	76.30 ± 0.35^{a}	20.56 ± 0.56^{de}
	Kolasib	66.50 ± 2.35^{b}	16.27 ± 0.94^{gf}
	Bilkhwalthlir	62.26 ± 1.77^{bc}	18.10 ± 0.08$^{d–f}$
	Buchangphai	58.94 ± 4.66^{cd}	21.60 ± 0.52^{cd}
Aizawl	Tuirial zau	27.53 ± 1.16^{jk}	10.52 ± 0.37^{hi}
	Keifang	33.50 ± 1.41$^{h–j}$	7.35 ± 0.89$^{i–k}$
	Shiphir	28.36 ± 1.06^{ij}	7.57 ± 1.49^{ij}
	Tlungvel	35.53 ± 0.69^{gh}	4.63 ± 0.47$^{j–m}$
Champhai	Khawzawl	55.42 ± 1.19^{d}	14.09 ± 1.70$^{f–h}$
	New Champhai	43.46 ± 1.25^{e}	15.68 ± 0.44^{gf}
	Zotlang	55.62 ± 0.35^{d}	21.66 ± 1.16^{cd}
	Vapar	56.55 ± 2.62$^{k–m}$	16.57 ± 3.55$^{e–g}$
Serchhip	Chekawn	22.67 ± 1.33$^{k–m}$	6.71 ± 0.99$^{i–l}$
	Bunghmun	62.51 ± 1.60^{cb}	1.57 ± 0.26^{m}
	Thenzawl	26.46 ± 0.60$^{k–m}$	3.04 ± 0.131^{lm}
	East Lungdar	22.62 ± 1.47$^{k–m}$	3.32 ± 0.32$^{k–l}$
Lunglei	Hnanthial	37.50 ± 0.45^{fg}	25.49 ± 0.65^{bc}
	Tuipui-D	25.32 ± 1.03$^{k–m}$	30.44 ± 1.16^{a}
	Vanhne	17.72 ± 1.18^{nm}	26.12 ± 1.83^{b}
	Tawipui S&N	57.86 ± 5.83^{cd}	15.04 ± 1.42^{gf}
Saiha	Meisavaih	45.52 ± 1.58^{e}	21.86 ± 2.05^{cd}
	Maubawk	55.61 ± 0.46^{d}	15.97 ± 2.96^{gf}
	Kalchaw-E	57.51 ± 2.23^{cd}	15.62 ± 2.66^{gf}
	Lungban	66.47 ± 0.57^{b}	15.13 ± 2.59^{gf}
Lawngtlai	Rawlbuk	30.22 ± 1.33$^{h–j}$	16.61 ± 2.03$^{e–g}$
	Sihtlangpui	42.55 ± 0.31^{fe}	10.12 ± 0.31^{hi}
	Chawnhu	12.53 ± 1.41^{no}	10.08 ± 1.21^{hi}
	Diltlang	22.52 ± 2.05$^{k–m}$	10.22 ± 0.97^{hi}
Mamit	West Serzawl	21.56 ± 0.55^{ml}	12.46 ± 2.09^{gh}
	Marpara	30.23 ± 0.98$^{h–j}$	4.52 ± 1.08$^{j–m}$
	West Pheling	25.14 ± 1.90$^{j–l}$	10.39 ± 0.34^{hi}
	Lengpui	11.77 ± 1.90^{o}	5.48 ± 0.99$^{j–m}$
	LSD	5.32	4.18
	Pr ≥ *F*	<0.0001	<0.0001

*Unidentified bark borer. [a]Standard error of the mean = SEM, means from 4 replicates, each of 40 tree bean tree/villages. [b]Means followed by different letters within the column are significantly different according to Fisher's least significance differences test ($P = 0.05$).

Most diseased trees showed signs of bark borer damage and emitted trash from small holes (Figure 4 *d*, *e* and *g*). The hypothesis indicated that these bark borers may play a vital role in spreading the disease within the villages, districts, states and most importantly transboundary (from neighbouring countries), through exchange of planting materials. Secondary infestation by trunk borer and long horn borers was observed when the dried and old trunks were chopped off. Similar signs and symptoms of the disease were reported from different tree species like mango[16] from Pakistan, and pear[25,26] and jatropha[27] from India.

Age wise susceptibility

One thousand six hundred (1600) tree bean trees were grouped into four different ages, i.e. 1–10, 11–20, 21–30 and >31 years based on the grower's feedback, to study the susceptibility level against the sudden death or decline. The highest severity (62.78%) was observed in the age group of 1–10 years, to the tune of 917 (57.32%) trees of the total trees examined. This age was considered to be the most susceptible age to the decline disease. The least severity (20.63%) was recorded in 80 (5.02%) trees in the age group of >30 years. On the other hand, disease prevalence was recorded on tree bean (Figure 5) in the age group 11–20 years comprising 401 trees of the total 1600 trees examined[17]. Insect diversity between freshly wilting and dead trees significantly differed, and secondary infestation with wood boring borer, long horn borer, the bark borer was dominant in dead trees[11].

Fungal isolation and morphology

In this study, a total of 24 isolates obtained from different sources were used. The predominant fungi isolated on potato dextrose agar (PDA) was *B. theobromae*, *P. disseminata* and *P. guepeni* from all locations of Mizoram. Comparatively, higher frequency of *B. theobromae* occurrence was recorded than *P. disseminata* and *P. guepeni*. During most of the study, mycelial growth of *B. theobromae* dominated over other pathogenic fungi on PDA media (Figure 6). *B. theobromae* was isolated from

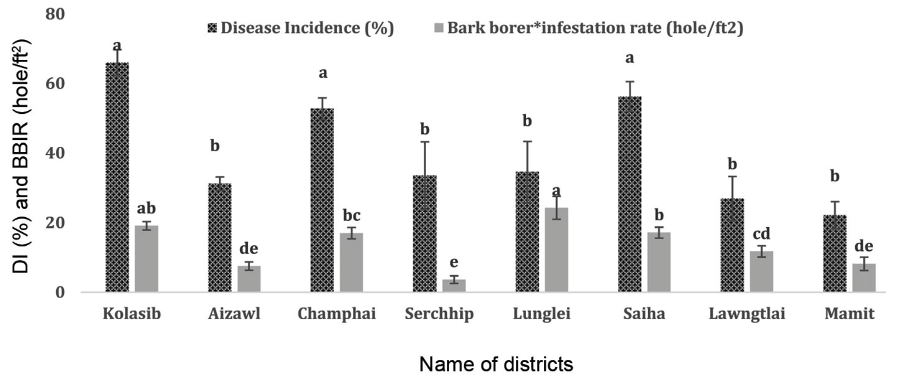


Figure 3. Disease severity (%) and bark borer infestation rate (hole/ft^2 log) of sudden death or decline in disease of tree bean trees in different districts of north eastern hill region, Mizoram.

Table 3. Pathogenicity test and symptom severity due to infection with isolates of *P. disseminata*, *B. theobromae* and *P. guepini* on one-year-old tree bean seedlings

Fungal species	Symptom severity[a,b]		
Isolates	Oozing (number) ± SE	Wilting (after 7 months) ± SE	Lesion length (mm) ± SE
Pestalotiopsis disseminata MZ1*	1.05 ± 0.41[b]	17.69 ± 5.93[bc]	1.63 ± 0.31[bc]
Pestalotiopsis disseminata MZ2	1.00 ± 0.41[b]	10.79 ± 1.61[c]	1.78 ± 0.05[bc]
Botrydiplodia theobromae MZ6	3.09 ± 0.71[a]	90.04 ± 4.44[a]	11.65 ± 1.36[a]
Botrydiplodia theobromae MZ9	3.52 ± 0.41[a]	87.44 ± 2.65[a]	10.79 ± 0.59[a]
Pestalotiopsis guepini MZ5	1.00 ± 0.41[b]	21.37 ± 1.34[b]	2.52 ± 1.16[b]
Pestalotiopsis guepini MZ3	0.50 ± 0.29[b]	12.73 ± 1.12[bc]	1.75 ± 0.32[bc]
Healthy seedling (control)	0.00 ± 0.00[b]	00.00 ± 0.00[d]	0.00 ± 0.00[d]
Isolates	F = 7.80, P = <0.0001[c]	F = 147.97, P = <0.0001[c]	F = 43.9, P = <0.0001[c]
LSD	1.24	9.14	2.15

*MZ Mizoram. [a]Standard error of the mean = SE, means from 4 replicates, each of 5 young seedling of tree bean tree; [b]Means followed by different letters within the column are significantly different according to Fisher's least significance differences test (P = 0.05); [c]P values of fixed effects associated with F test.

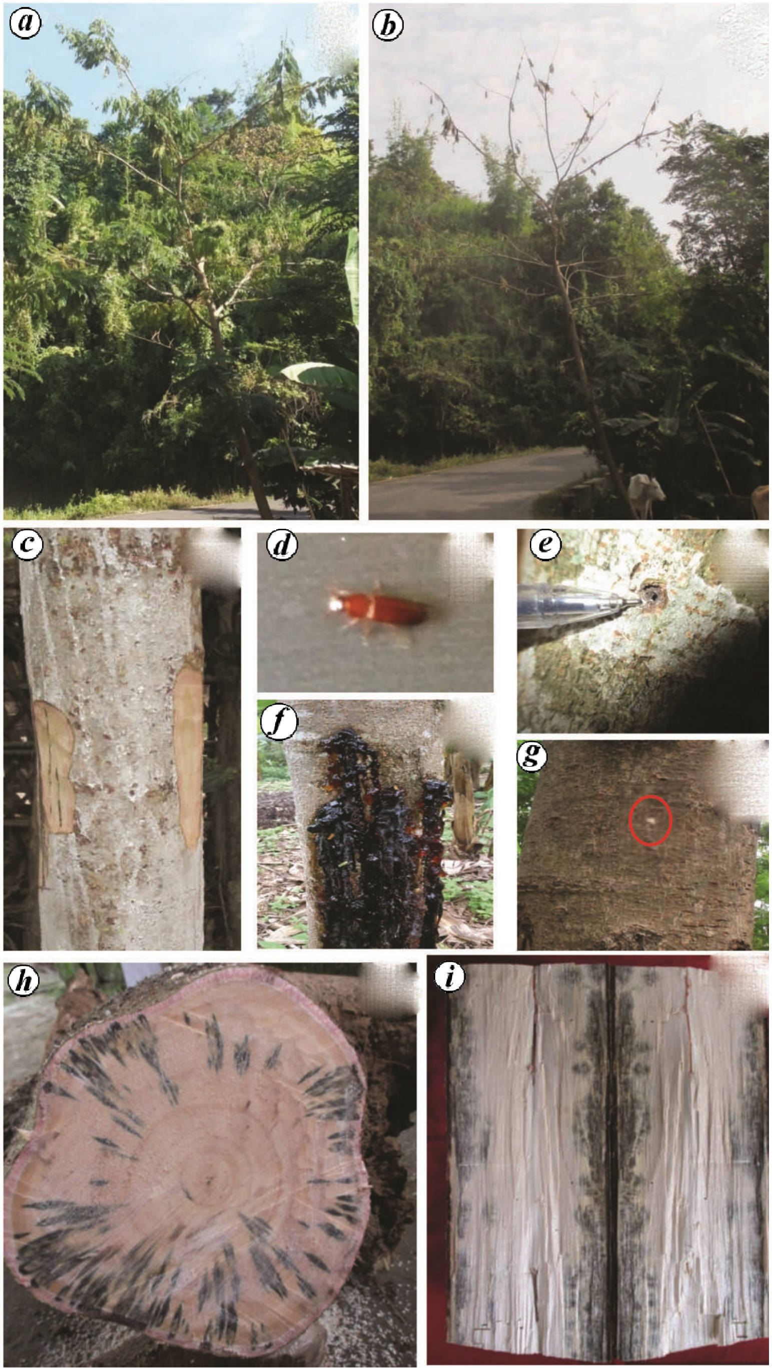


Figure 4. Natural signs and symptoms of tree bean sudden death or decline. ***a***, Initial wilting; ***b***, wilting and excessive leaves shedding and sudden death; ***c***, half infected and half healthy trunk; ***d***, ***e***, unknown bark borers and holes made by them; ***f***, excessive gummosis; ***g***, trash emission from holes; ***h***, black streak at crown cross section of infected tree trunk, vascular discoloration; ***i***, black streak at longitudinal section of infected tree.

the margins of discoloured wood tissues, trash, gum, insect, while *Pestalotiopsis* spp. was isolated from the collar portion of the tree trunk. A small bark borer was found most frequently on diseased trees and collected for isolation of fungi. The fungal isolation studies from the bark borer showed its frequent association with *B. theobromae*. The fungi, *P. disseminata* and *P. guepeni*, appeared at a relatively lower frequency on PDA isolation media. Colonies of *B. theobromae* on PDA were initially greyish-white in colour which turned into dark-greyish-black after 15 days with a sporulation (Figure 7). Conidia were 20–28 × 11–13 μm, hyaline, aseptate, sub-ovoid to ellipsoid, with broadly rounded apices and later became dark brown, single-septate and thick-walled.

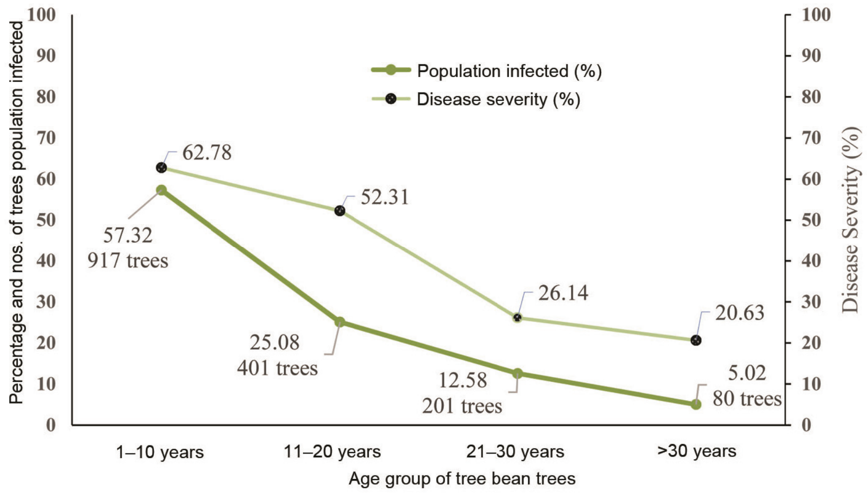


Figure 5. Per cent (%) and total plant population (number) infected and disease severity (%) of sudden death or decline disease of tree bean trees in Northeastern hill region, Mizoram.

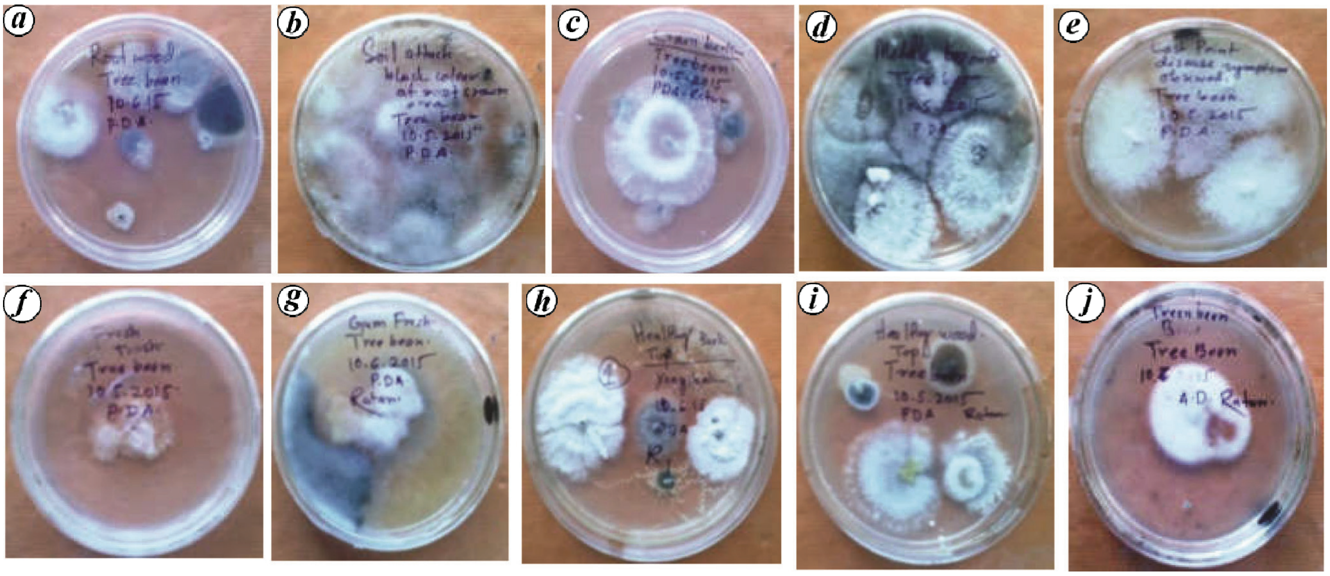


Figure 6. Isolation of different pathogens associated with sudden death or decline disease of tree bean from various part of plant. ***a***, Soil adhere to the tap root; ***b***, tap rot; ***c***, tree crown; ***d***, middle part of tree trunk (bark); ***e***, middle part of tree trunk (wood portion); ***f***, top of tree trunk (bark); ***g***, top of tree trunk (wood portion); ***h***, free gum; ***i***, fresh trash emission from borer hole; ***j***, from insect borer.

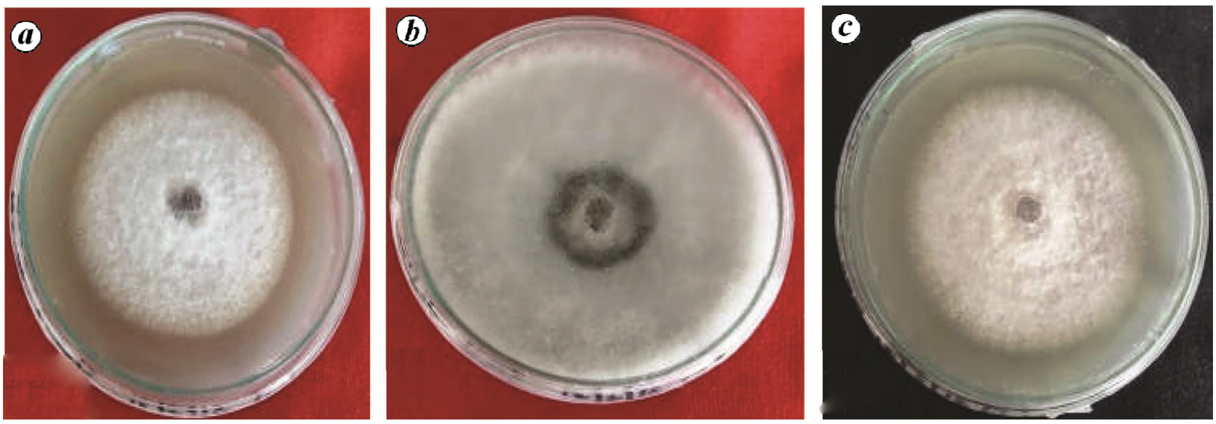


Figure 7. Morphological characteristics of the front sides of colonies of the different fungal species grown on PDA associated with sudden death or decline disease of tree bean. ***a***, *Pestalotiopsis disseminate*; ***b***, *Botrydiplodia theobromae* (most frequently isolated from different parts of tree bean trees); ***c***, *Pestalotiopsis guepini*.

Representative cultures were also identified (*P. disseminata* (Id: 9881.2); *B. theobromae* (Id: 9881.3) and *P. guepini* (Id: 9881.4)) and deposited at ITCC. Conidial morphology and cultural features of the isolates closely agree with the morphological description[27–30].

Pathogenecity and aggressiveness test

Two isolates of each pathogen, *B. theobromae*, *P. disseminata* and *P. guepini*, were used for conducting the pathogenicity test. After seven months of inoculations, disease symptoms, i.e. wilting, oozing (gum) and lesions of black streaks developed, which showed significant differences among all isolates (Table 3). Initial wilting (after two months) appeared high enough on plants inoculated with *B. theobromae*, which became more drastic after seven months as compared to *P. disseminata* and *P. guepini.* Maximum oozing (number of gummosis) and black streak symptoms were exhibited when the seedlings were inoculated with isolates of *B. theobromae* (Table 3). The plants artificially inoculated with *B. theobromae* were re-isolated in high frequencies. Control plants did not exhibit any of the fungus growth and symptoms. The pathogenicity test demonstrated that the most frequently isolated fungi, *B. theobromae*, induced symptoms of sudden death or decline in tree bean after inoculation to healthy seedlings (Figure 6). Thus, the two isolates of *B. theobromae* might be considered to be the most virulent since they produced the most severe symptoms. The *Pestaliopsis* species showed least virulence. This test was conducted and confirmed by Koch's postulates[31]. *B. theobromae* proved to be more pathogenic in inducing disease symptoms[32]. This conclusion is also based on the consistent isolation of *B. theobromae* from the stem of infected tree bean trees, insect, trash and gum. Therefore, these fungi might be contributing to the symptoms and development of sudden death of tree bean trees, which include bark splitting, rotting of stems, vascular discolouration and gummosis. This disease aggressiveness by *B. theobromae* has been reported from different parts of the world including India in the case of mango[33] and common tea[34].

The association of bark borers with diseased tree beans may possibly act as a vector of spores of *B. theobromae* on tree beans. The rapid spread of sudden death or decline of tree bean disease across all the tree bean growing areas of North Eastern region of India showed the possibility of involvement of insect vector. A bark borer which is encountered in a freshly dead tree makes its role as a putative vector which needs to be addressed in further studies. In spite of being a high value tree vegetable crop[35], tree bean trees are becoming increasingly vulnerable to infection due to improper irrigation, root injuries either by termites or ploughing and lack of phytosanitary measures in the tree bean plantation. Therefore, it is necessary to develop an integrated pest disease management system for tree bean production, to minimize the risk of damage due to the decline disease. This work represents the first report of *B. theobromae* as the causal agent of the sudden death or decline of tree bean in the north-eastern region of India.

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