Progress Report for DBT Twining R&D Project Work
Progress Report for the Year (2015-18)

Towards identification, isolation and characterization of *Exobasidium vexans* strains and their pathogenic determinants/effectors, from Blister blight infested tea-plantations of Assam and development of a future road-map for effective management practices.

Sanction Order: BT/247/ NE/TBP/2013 dated. 25.03.2015
(Year: 2015-18)

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Progress Report for R&D Projects (2015-18)

Section A: Project Details

A1. **Project Title:** Towards identification, isolation and characterization of *Exobasidium vexans* strains and their pathogenic determinants/effectors, from Blister blight infested tea-plantations of Assam and development of a future road-map for effective management practices.

A2. **DBT Sanction Order No. & Date:** BT/427/ NE/TBP/2013 Dated. 25.03.2015

A3. **Principal Investigator (Parent Institute):** Dr. E. Kalita, Department of MBBT, T.U.

Co-Investigator (Parent Institute): Dr. P. Deb, Department of Physics, T.U.

A4. **Institute:** Tezpur University (Central University), Tezpur.

A5. **Principal Investigator (Collaborating Institute):** Dr. Praveen K. Verma, NIPGR.

Co-Investigator (Collaborating Institute): Dr. Debasis Chattopadhyay, NIPGR.

A6. **Institute:** National Institute of Plant Genome Research, New Delhi.

A7. **Address with contact nos. (Landline & Mobile) & E-mail:**

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A6. **Total cost:** Rs. 90.27 lakhs

A7. **Duration:** Three (03) Years

A8. **Approved objectives of the project:**

Period of study	Achievable targets
6 Months	Phase 1 Isolation, Identification and establishment of pure culture
12 Months	Phase 2 Isolation, Identification and establishment of pure culture Phase 1 transcriptome profiling and identification of Key candidate effectors
18 Months	Phase 3 Isolation, Identification and establishment of pure culture Phase 2 transcriptome profiling and identification of Key candidate effectors
24 Months	Phase 3 transcriptome profiling and identification of Key candidate effectors Phase 1 Gene-Knockouts/RNAi generation and bioassay
30 Months	Phase 4 transcriptome profiling and identification of Key candidate effectors Phase 2 Gene-Knockouts/RNAi generation and bioassay Phase 1 Identification of Pathogenic effectors and development of future roadmap
36 Months	Phase 3 Gene-Knockouts/RNAi generation and bioassay Phase 2 Identification of Pathogenic effectors and development of future roadmap

A9. Specific Recommendations made by the Task Force (if any). : NA

DBT has granted sanctions for our project proposal entitled "*Towards identification, isolation and characterization of Exobasidium vexans strains and their pathogenic determinants/effectors, from Blister blight infested tea-plantations of Assam and development of a future road-map for effective management practices*" vide letter no. **BCIL/NER-BPMC/2015-230 dated 08.04.2015 through the sanction (BT/247/NE/TBP/2013 Dated. 25.03.2015)** being the 1st instalment for the implementation of the project.

Section-B: Scientific and Technical Progress

B1. Progress made against the Approved Objectives, Targets & Timelines during the Reporting Period:

B1.1. Introduction:

Tea (*Camellia* spp.) is a major cash crop and continues to be one of the most popular beverages consumed worldwide due to its attractive aroma, pleasant taste, and numerous medicinal benefits. In India, the annual yield of tea accounts for an estimated 31% of the world production and constitutes 20% of the global production area **(Mur et al. 2015, Sowndhararajan et al. 2012)**. Given the economic importance of tea, the factors that impose a threat to its quality and yield are of great significance. Among the different factors that affects tea production, blister blight caused by the pathogenic fungus *Exobasidium vexans* is the most serious foliar tea disease, imposing a total yield loss as high as ~43% of the cultivated area. In North-East India, the disease occurs predominantly in the hilly regions of Darjeeling and Assam and results in a crop loss of up to ~24% **(Bhorali et al. 2012)**.

E. vexans is an obligate biotrophic fungus that mainly affects young succulent leaves and harvestable tender shoots of the plant, causing enormous crop loss. The infection is mainly favored by cloudy wet weather and high humidity **(Jeyaramraja et al. 2005)** and the primary stage of infection shows small pinhole size spots in young tender leaves. With the growth of the leaf, the area of the infected spot also increases and results in a concave shaped depression on the dorsal leaf surface. Simultaneously, the ventral side of the leaf becomes convex to form the typical blister lesion. After the release of fungal spores, the blisters become white and velvety in appearance which subsequently leads to leaf curling and necrosis. The pathogen completes its entire life cycle in tea and is not known to have any alternate host **(Premkumar et al. 2008)**.

Various ways for managing blister blight have been in practice, of which the use of chemical fungicides is mostly prevalent. However, these chemical agents are known to be hazardous also towards non targeted organisms and their prominent usage possesses a severe threat to the environment **(Bhorali et al. 2012)**. Thus, there has always been an impending search for alternatives such as avirulent pathogens, plant growth

promoting rhizobacteria, biotic and abiotic elicitors etc. for the induction of acquired resistance in host plants, thereby reducing the severity of blister blight infections. However, for the production of an effective biocontrol agent, the study of the pathogen and its host in broad sense is of utmost necessity. Numerous reports exist that describe the defense mechanism as well as defense genes expressions of the host plant w.r.t. blight infections caused by *E.vexans*. Bhorali et al. (2012) have reported that *E. vexans* rapidly modulates the expression of a large repertoire of defense genes in *Camellia* spp. along with the stimulation of signal transduction and associated modulation of other biochemical pathways. In addition, a significant increase in the activities of defense enzymes like phenylalanine ammonia lyase, peroxidase and β-1, 3-glucanase was found on elicitor treatments in tea leaves challenged with the pathogen **(Ajay et al. 2009)**. Treatments with chemical elicitors ASM (acibenzolar-S-methyl benzo-[1,2,3]- thiadiazole-7- carboxylic acid S-methyl ester) and salicylic acid are reported to result in reduced severity of blister blight disease in nursery plants on challenged with the pathogen. **(Ajay et al. 2009)**. Moreover, reports have also stated higher amounts of epicuticular wax and increased thickness of cuticle/epidermal layer of the resistant tea clone SA-6 against blister blight disease. **(Jeyaramraja et al. 2006)**.

Although, a few studies have been carried out on the host plant resistance, no reports have been stated so far about the pathogen *E.vexans* in relation to its interaction with the tea plant. Thus, the comprehensive study of the pathogen and its mechanism of infection may likely to help in the development of novel and efficient approaches in controlling this devastating disease while bringing about a significant increase of the quality and quantity of tea.

B1.2. The Study Area: Due to the relative abundance of infested areas, the Happy Valley Tea Estate, Ananda Tea Estate and Pathalipam Grant was chosen as the site for the collection of blister blight infected samples. The infection starts during the month of May-June and lasts up to the month of August. During these months the atmospheric humidity was found to be substantially high and is known to favor the infection and spread of the blister blight fungus in the cultivated tea plants.

B1.3. Methodology: To carry out the study, a systematic methodology was applied. The tea leaf parts, infected with blister blight were collected aseptically and inoculated in petridishes containing growth medium. The inoculated samples were allowed to germinate for mycelial growth and were subsequently examined for spore morphology using microscopic technique. Pure cultures of *Exobasidium vexans* were established via single spore culture method and were maintained in czapek dox agar (CDA) media with continuous sub-culturing at every 72-96 h. The obtained pure culture was used for the downstream experiments detailed in the next section.

B1.4. Achieved Objectives in 2015-18:

B1.4.1. Collection of Blister blight infected tea leaf samples

In the first phase of the study, Blister blight infected tea leaf samples were collected from various sectors of Happy Valley Tea Estate, for the isolation of *Exobasidium vexans* strain (Fig. 1).

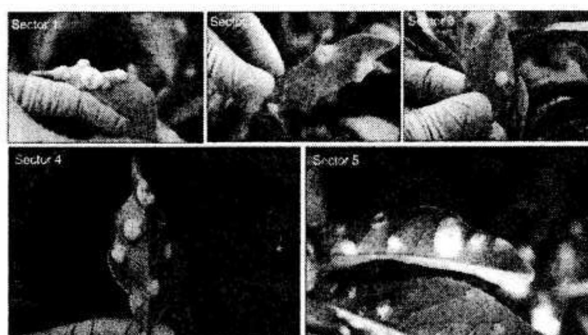


Fig. 1. Tea leaves with blister blight lesions

In the second phase of the study, 11 tea estates were surveyed for occurrence and collection of samples from blister blight infected tea plantations. These surveyed areas included tea estates of Lakhimpur, Jorhat and Dibrugarh districts (Table 1). Of the enlisted areas, blister blight infection was only found in the tea estates of Lakhimpur district. This could be due to the fact that the existing climatic conditions of Lakhimpur district, during the survey period, consisting of low temperature, high humidity and low sunshine are favorable for the blister blight infection **(Ajay et al. 2009)**. In addition, Lakhimpur district receives high rainfall, as it is bordering areas near Arunachal Pradesh that typically have low sunshine and high humidity, which favors the blister blight infection. Fig.2 shows the representative images of blister blight infected tea leaves collected from blister blight affected tea plantations of Lakhimpur District, Assam. Fig. 3 represents the map data showing the geographical locations of the visited tea estates.

Table 1. Tea Estate visited for collection of blister blight infected tea samples

District	Tea Estate	Latitude	Longitude	Month	Temperature (°C)	Humidity (%)
Lakhimpur	**Koilamari Tea Estate**	27.199544	95.112074	April	19-28°C	0.81%

	Ananda Tea Estate	27.270202	95.126658			
	Pathalipam grant	27.460672	94.238178			
Jorhat	**Sangsuwa Tea Estate**	27.266016	95.275911	May	20-36°C	0.85%
Dibrugarh	**Mancotta Tea Estate**	27.206587	95.071278	May	21-34°C	0.74%
	Kenduguri Tea Estate	27.438446	94.917752			
	Rajgarh Tea Estate	27.369646	95.281286			
	Salmari Tea Estate	27.252111	95.303614			
	Desam Tea Estate	27.383772	95.341369			
	Balijan Tea Estate	27.311771	94.056975			
	Dulia Tea Estate	27.452734	94.230063			
	Jaloni Tea Estate	26.674146	94.088497			

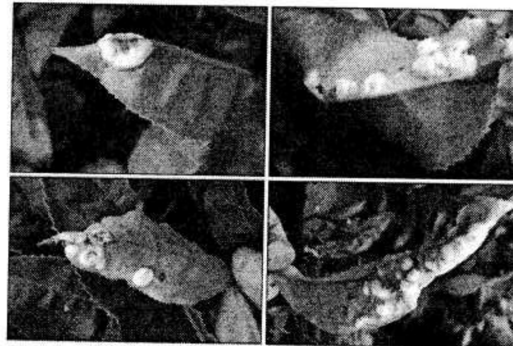


Fig. 2. Blister blight lesions in the leaves of tea plant

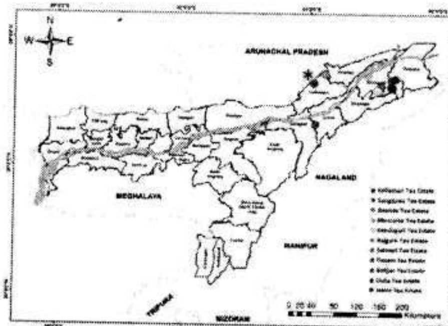


Fig. 3. Map data showing geographical locations of visited tea estate

B1.4.2. Isolation of *Exobasidium vexans* from blister blight infected tea leaf samples

The infected part of the leaf was cut with the help of a sterile cork borer and transferred into petridishes containing czapek dox agar (CDA) media (Fig. 4 a). After a period of 5 days, mycelial growth for the fungal pathogen was observed. The mycelia were collected from the surface of the petridish with the help of a sterile loop and transferred onto new petridishes containing CDA using a streak plate approach (Fig. 4 b). The streaked plates were allowed to incubate at room temperature under dark conditions. After a period of incubation of 72-96 h the plates were used for the preparation of fungal spore suspensions. The fungal spore suspensions were subsequently spread onto sterile petridishes for single spore culture (Fig. 4 c).

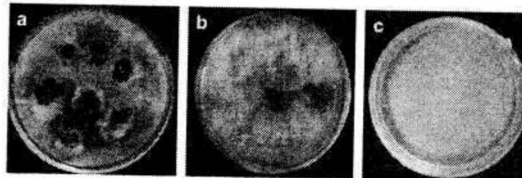


Fig. 4. Petridishes showing inoculation of infected leaf part (a), growth of *E. vexans* in czapek dox agar media (b) and single spore culture of *E. vexans* (c)

B1.4.3. Identification of *E. vexans* based on the study on spore morphology

The identification of *Exobasidium vexans*, from the infected tea leaves, was carried out by the examination of the fungal spore morphology for the isolated samples using Light microscope and Fluorescence microscope (NIPGR).

The microscopic examination of the spore suspensions was carried out using Cotton blue stain and was visualized under light microscope. The Fluorescence microscopic examinations were carried out using Calcofluor white stain (NIPGR). From the light micrographs, the basidiospores of *Exobasidium vexans* were found to be ellipsoid to ovoid in shape (Fig. 5).

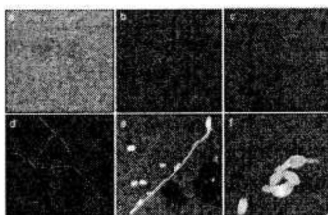


Fig. 5. Light micrograph images showing Spores of *Exobasidium vexans* at 10X magnification (a), germ tube growth (b,c), and anastomosis (d) at 40X magnification and Fluorescence micrograph images [Performed at NIPGR] showing germ tube growth (e,f) at 60X magnification

After completing the study of spore morphology of *E. vexans* using CFW (calcofluor white) stain, the study of *in vitro* germination of *E. vexans* basidiospore was carried out to study the growth of *E. vexans*. To study the process of *in vitro* germination of basidiospores, a defined area of the slide was covered using agar and a drop of the basidiospore suspension prepared from pure culture was added on to the agar surface. These slides were then incubated for different time intervals at 28°C and the spore germination was observed under a light microscope. The dimensions of the spore were measured using the Image J software. Fig. 6 a show the light micrographs of fully developed *E. vexans* basidiospores, which were ellipsoid to ovoid in shape and somewhat curved, with a slightly tapered end. The average size of the basidiospores was 6.03 - 19.37 X 2.3 – 5.8 µm. The basidiospores germinating on the agar surface, 6h post incubation was seen to have the germ tubes emerging from either one or both ends of the basidiospore (Fig. 6 b, c). The basidiospores were aseptate during the initial stages of germination, which was followed by the appearance of 1-4 transverse septa during the later stages. This process of septation and the emergence of germ tube from the basidiospores, precisely matches the detailed description of the *E. vexans* basidiospore germination, reported by Gadd and Loos in 1949. Further, the formation of fungal hyphae was observed after an incubation period of 16h which differentiated into branches with anastomosis between them (Fig. 6 d, e). The morphology of the fungal hyphae was also observed with WGA (wheat germ agglutinin) stain under fluorescence microscope to observe the fungal cell wall (Fig. 6 f).

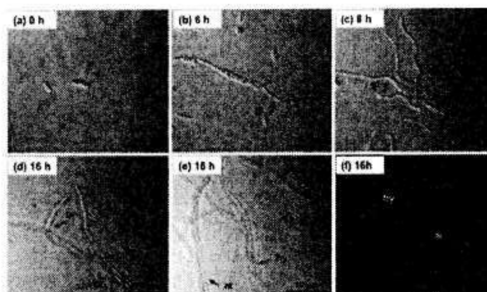


Fig. 6. *In vitro* germination of basidiospore of *E. vexans*, 40X (gt – germ tube, br – hyphal branching, at – anastomosis)

B1.4.4. Identification of isolated fungal strains of *Exobasidium vexans* by sequencing Internal Transcribed Spacer (ITS) region, phylogenetic analysis, divergence time estimation and rRNA secondary structure prediction

After the morphological identification of *E. vexans* the molecular based identification was carried out to develop DNA barcode for *E. vexans* to overcome the difficulty related to cryptic speciation of the large phylum basidiomycota. The nuclear ribosomal internal transcribed spacer region of fungus is considered as DNA barcode for the molecular based inter- and intraspecific identification of fungus and to carry out its phylogenetic analysis. It consists of the 18S, 5.8S and 28S rRNA genes with the two internal transcribed spacers ITS1 and ITS2 across the 5.8S rRNA. Although various universal primer has been reported to amplify the ITS region of fungus, no species-specific primers have been reported for the identification of *E. vexans* till date. In this context, species specific primer for *E. vexans* was designed to develop DNA barcode for the identification of *E. vexans*. DNA extraction was carried out from fungal isolates after grown for 5–7 days on PDA agar media using phenol-chloroform extraction using SDS lysis buffer and ethanol precipitation. The ribosomal internal transcribed spacer (ITS) rDNA region was amplified using Phusion® High-Fidelity DNA Polymerase (Thermo Fisher Scientific). Three primer pairs are used in this study (Table 2). Primer pair 1(ITS1F-ITS4B) is a universal primer primarily used for amplification of the basidiomycetes ITS region. The second primer pair (LSUF-LSUR) was designed from an *E. vexans* species-specific 28srRNA sequence available online (Accession No: AB180380) for validation of the ITS region at the species level. The third primer combination used was the universal forward primer (ITS1F) and the *E. vexans* specific reverse primer (LSUR). This primer pair was used to facilitate the amplification of a larger fragment from the *E. vexans* ITS region, compared to that

obtained by the universal primer specific for basidiomycetes ITS region (ITS1F-ITS4B). The positive PCR products from these amplifications were cloned with CloneJET PCR Cloning Kit (Thermo Fisher Scientific) and sequenced using Sanger Sequencing (Fig. 7). Isolate 1 (Happy Valley tea estate) and Isolate 2 (Ananda Bagan tea estate) was used for the current study. The MEGA X software was used for the maximum parsimony (MP) and maximum likelihood (ML) phylogenetic analysis of the *E. vexans* sequences obtained in this study, in relation to the available sequences of other species showing homology >80% in blast analysis. The chosen substitution model for the dataset was the Tamura-Nei model with a discrete gamma distribution (T92+G). Mr. Bayes software was used for the BI phylogenetic tree construction. The maximum parsimony method was applied using a subtree-pruning-regrafting model. Bootstrap values were obtained from 1000 bootstrap replicates and all gap/missing sites were used for the construction of the phylogram. During BI analysis using MrBayes 3.2.7, four Markov chains were run for 10,000,000 generations and sampling trees was carried out every 1000 generations with 25% burn in **(Ronquist and Huelsenbeck, 2003)**. The MCMC tree constructed with the remaining 75% was visualized in FigTree v1.4.4 **(Rambaut, 2018)** and Bayesian posterior probabilities (BPP) of the clades were calculated. Log files were viewed with Tracer v1.7.1 and were assesses foreffective sample sizes (ESS) of >200 and satisfactory mixing of the MCMC chains **(Rambaut and Drummond, 2007)**. Molecular dating for divergence time estimation of *E. vexans* was analyzed with several individual strains per species of genus *Exobasidium* using the BEAST v2.6.0 (Bayesian Evolutionary Analysis Sampling Trees) software package **(Bouckaert et al., 2019)**. Calibration of the divergence of *E. vexans* was carried out with a normally distributed prior for a mean of 582.5 Mya with a standard deviation of 50 Mya. For the dating analyses, Yule speciation prior set was applied for the strict molecular clock in BEAST to estimate the divergence time. MCMC analysis mediated the calculation of posterior distributions of parameters for 10 million generations with 10% burn-in. The .xml BEAST input files were generated using BEAUti within BEAST and maximum clade credibility summarized the posterior distributions using the program Tree Annotator v2.6.0 with the posterior probability limit of 0.8. FigTree v1.4.4 was used to visualize the resulting tree and the chain convergence was established using Tracer v1.7.1 **(Rambaut, 2018, Rambaut and Drummond, 2007)**.

The genomic DNA (gDNA) of the *E. vexans* isolates (1 & 2) were extracted, checked on an agarose gel, quantified and then, appropriately diluted for PCR amplifications. Subsequently, positive amplicons from PCR amplifications were cloned and sequenced. This was followed by the comparison of the obtained ITS region of the isolates 1 & 2 with the available ITS sequences using the NCBI blastn tool **(Altschul et al., 1990)**. The complete ITS region sequence of ~1150 bp, developed here is longer compared to other available ITS region sequences for *E. vexans* in the database. Altogether, 94 ITS region sequences showed high homology (>80%) with our strains including multiple strains of an individual species which were considered for further phylogenetic analysis.

The MP phylogenetic tree based on ITS rRNA region indicates a 98% identity of isolates with *E. vexans* strain CBS 247.52 (Fig. 8 a). Here, the most parsimonious tree with a length of 4786 has a consistency index of 0.650985, retention index of 0.775942, and composite index of 0.635127 for all sites and parsimony-informative sites. Identifying the isolated strains to be *E. vexans* is well supported by ML analysis and has a bootstrap value of 81% with *E. vexans* strain CBS 247.52 (Fig. 8 b). As evident from the BI phylogram, the identity of isolates as *E. vexans* strain is corroborated from the 100% Bayesian posterior probability with the *E. vexans* (CBS 247.52) strain derived from database (Fig. 8 c). The dataset from BI analysis yields the best-sampled tree with a log-likelihood around a mean of-19163.959. During the BI analysis, effective sample size (ESS) measurement of MCMC chain length were calculated taking into account the autocorrelation of the chain in successive steps. These ESS values when viewed from the log files with Tracer v2.6.0 showed that ESS ≥ 200 thereby confirming the satisfactory mixing of the MCMC chains (Table 3). During the MCMC analysis, sufficient burn in was summarized from the efficient mixing of the trace plots of the log-likelihood and split probabilities comparison throughout the independent runs. A Bayesian posterior probability ≥ 95% is considered to be an accurate estimator of clade identity which also corresponds to Bootstrap value ≥ 70% **(Hillis and Bull, 1993, Alfaro et al., 2003)**. As such, a BI based phylogenetic reconstruction is preferred over ML as Bayesian approach displays the posterior distribution of tree topologies. Thus, the sequence identity of isolates (1 & 2) as *E. vexans* (CNS 247.52) is well supported by the three phylogenetic methods (MP, ML & BI) with the standard level of clade confidence. Thereby we could generate *E. vexans* specific annotable DNA barcode capable of speciation which has shown significant homology with the existing *E. vexans* sequence in all the three (MP, ML & BI) phylogenetic algorithms.

To estimate the divergence time of *E. vexans* within the genus *Exobasidium* **(Bouckaert et al., 2019)** the ITS datasets were set as single partitions with unlinked substitution and molecular clock models. Also, the HKY (Hasegawa, Kishino, and Yano) substitution model and a strict molecular clock model was employed for the molecular dating analysis. An annotated maximum clade credibility tree was obtained and its analysis yielded effective sample sizes of >200, the recommended threshold for all relevant parameters, representing the adequate sampling of the posterior distribution. A 400 million-year-old fossil *P. devonicus* was the basis for the calibration of divergence for genus *Exobasidium*, which is usually favoured for molecular clock analysis of basidiomycetous fungi that uses divergence time between Basidiomycota and Ascomycota **(Chen et al., 2015)**. The phylogram representing the molecular clock analysis of *E. vexans* within genus *Exobasidium* is shown in Fig. 9. The numerical values in the individual node represents the node age and blue node bar indicates the 95% HPD (Highest Posterior Density) in the phylograms. Chronograms derived from molecular clock dating suggests that the diversification of the genus *Exobasidium* began approximately in the Cambrian period of Paleozoic era. Isolate 1 of *E. vexans* was found to have an individual mean node age of 340.89 Mya (247.17- 438.83 Mya; 95% HPD) which indicates the divergence period was in the Mississippian period of the Paleozoic era. The individual node age of the *E. vexans* isolate 2 was 45.8 Mya (31.48 - 57.75 Mya; 95% HPD) which is more recent in the *Exobasidium* lineages, evolving during early Eocene epoch of the Cenozoic era. The Phylogenetic analyses indicated that isolate 1 clustered with *E. vexans* (MH857014) while isolate 2 clustered with *E. woronichinii* (AB180680.1). While comparing the basidiospore morphology of isolated fungal spores, *E. vexans* and *E. woronichinii* basidiospores appear to share similar spore morphology. However, the divergence time estimation has now established this to be result of cryptic speciation.

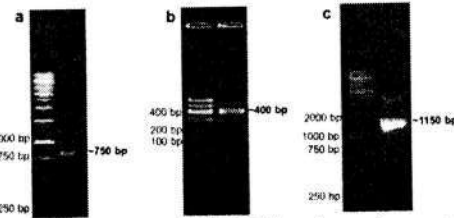


Fig. 7. Gel Electrophoresis Profile of amplicon product with gDNA using primer pair ITS1F-ITS4B (a) LSUF-LSUR (b) and ITS1F-LSUR (c)

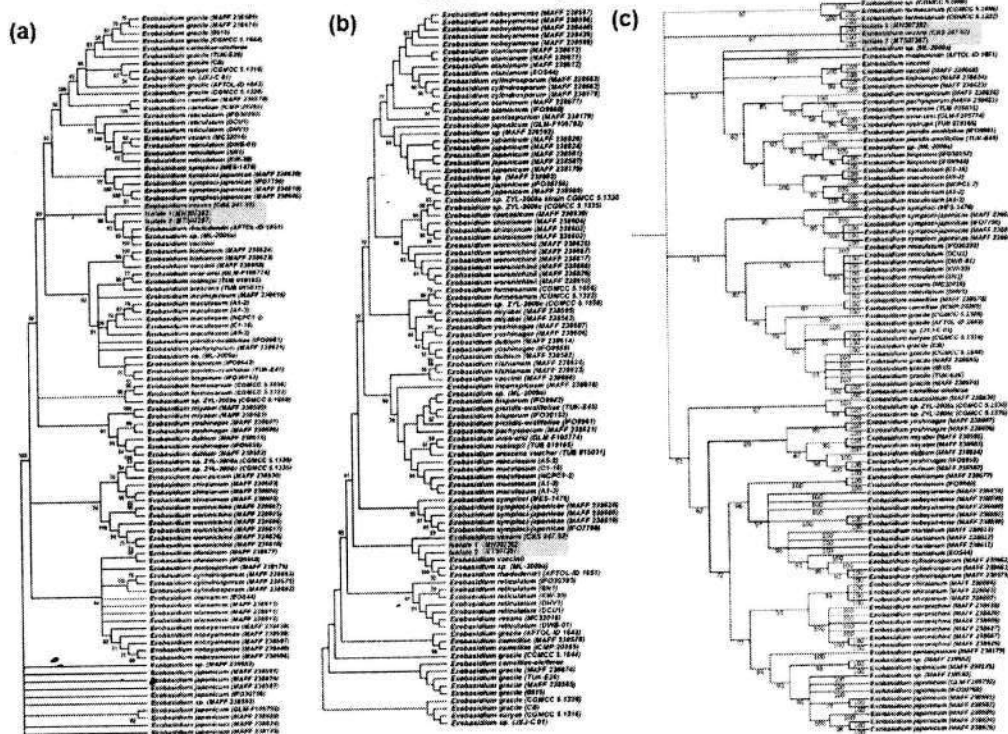


Fig. 8. Phylogenetic consensus tree for the 94 strains and isolates 1 & 2 inferred from the Maximum 183 Parsimony (MP) (a), Maximum Likelihood (ML) (b) using MEGA X [The numbers in the node of the8 184 phylogram indicate bootstrap values] and Bayesian Inference (BI) (c) using Mr. Bayes [The numbers in the 185 node of the phylogram indicate posterior probability values]

Table 3. Effective Sample Size (ESS) values for traces of log files

Statistics	ESS
Posterior	225
Likelihood	329
Prior	399
TreeLikelihood.ABEV	329
TreeHeight	1753
MutationRate	-
CalibratedYuleModel	400
BirthRateY	516
ClockRate	400
logP(mrca(basidiomycota))	3216
mrca.age(basidiomycota)	1753

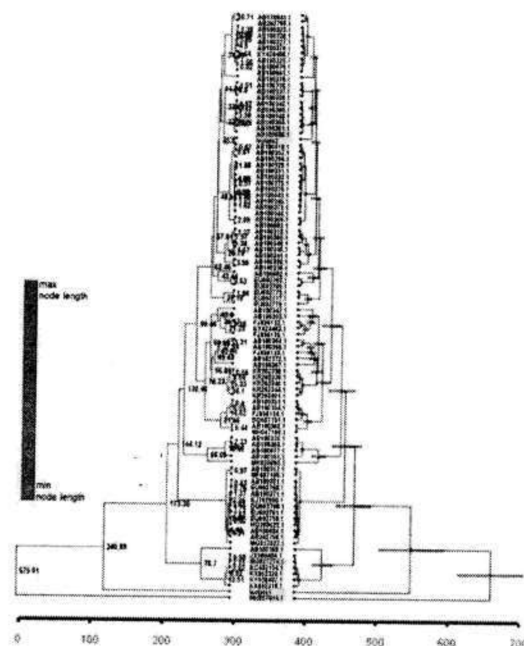


Fig. 9. Bayesian consensus Phylogram obtained by molecular clock dating using BEAST with node age (represented at individual node, left) and 95% HPD (blue node bar, right).

The ITS2 region annotation and the secondary structure prediction was performed using the web server http://its2.bioapps.biozentrum.uni-wuerzburg.de. The structure with the minimum free energy was selected and for each structural domain, the number of helices, the total number of paired bases and unpaired bases in bulges and/or interior loops were investigated and compared. Additionally, the consensus secondary structure for all the annoted species in the phylogram was predicted by aligning ITS2 primary sequences and their individual secondary structures in 4SALE v1.7.1. Further, compensatory base substitutions (CBCs) were counted using CBC analyzer in 4SALE that has been reported to be a molecular indicator for species delimitation.

Table 4. Strains and the accession numbers and host of the nine annoted species

Sl no.	Taxon	Strain	Accession number	Host species
1	*E. camelliae*	ICMP:20285	MF687185.1	*Acca sellowiana*
2	*E. cylindrosporum*	MAFF 238177	AB245089.1	*Rhododendron macrosepalum*
3	*E. euryae*	CGMCC 5.1316	EU692759.1	*Rhododendron sp.*
4	*E. feruginae*	BPI:882571	JQ611710.1	*Rhododendron sp.*
5	*E. japonicum*	MAFF 238826	AB180681.1	*Rhododendron obtusum*
6	*E. pentasporium*	MAFF 238601	AB180335.1	*Rhododendron obtusum var. kaempferi*
7	*E. rostrupii*	-	FJ896132.1	*Vaccinium oxycoccos*
8	*E. vaccinii*	DB160d	KP322983.1	*Vaccinium sp*
9	*E. woronichinii*	MAFF 238826	AB180680.1	*Rhododendron brachycarpum*

The ITS2 rRNA region of the annoted *Exobsidium* species was used to generate the secondary structure (Table 5). The secondary structure with lowest thermodynamic energy was selected and ranged from -42 kcal/mol to -54 kcal/mol. The secondary structure of the ITS2 rRNA region for all the studied species from *Exobasidium* genus was found to share a similar four-helix folding pattern except for *E. cylindrosporum* which was found with three-helix fold structure. As seen in Table 5 the variation in the length of the primary sequences of ITS2 is minimal. Studies have reported that higher GC content characterizes the evolution of ITS2 region within a genus. In the current study the query sequence was found to be with the high GC content of 47.8%.

The consensus structure of the ITS2 region for all the annoted species was generated based on the sequence-structure alignment. Seventeen base pair positions were found to be 100% conserved in the consensus structure, across the annoted species, of which, three were in helix 1(9/58: T-G, 10/57: C-G, 24/39A-T), seven in helix 2 (64/102: G-T, 65/101: G-C, 66/100: G-C, 71/95: C-G, 74/92: G-C, 77/90: G-C, 78/89: T-A) and seven present in helix 3 (114/174: G-C, 115/173: C-G, 118/171: G-C, 124/165: C-G, 128/156: A-T, 132/152: T-A, 137/148: T-G). From the structural alignment study, two position in helix 2 were found to be conserved, 65/103: G-U in *E. japonicum*, *E. pentasporium*, *E. woronichinnii*, *E. vaccinii*, *E. ferrugineae*, *E. rostrupii*, *E. cylindrosporum* and 59/111: U-A in *E. camelliae* and *E. euryae*. Whereas, one position in helix 3 (117/180: A-U) was found to be conserved in most of the *Exobasidium* species. Further, CBC calculations carried out for delimiting species boundaries are detailed in table 6. According to the CBC hypothesis, presence of a single CBC between two species makes them different with a probability of ~93% while the absence of CBC indicates that they may be similar, with a probability of ~73% **(Wolf et al. 2013)**. From the CBC matrix (Table 6), a maximum of three CBC, included in both conserved and variable region, was found between two species. For the query sequence, absence of CBC was seen which implies to the fact that the isolated strain resembles to that of the listed *Exobasidium sp.*

Table 5: Characterization of ITS2 secondary structures of the annoted *Exobasidium* species and query

Organism	Sequence length (bp)	No. of Helix	Base pair formation in helices (%)	Loop length (unpaired bases)	ΔG (kcal/mol)	GC content (%)
E. camelliae	209	4	58.3	87	-50.9	41
E. cylindrosporum	206	3	53.3	92	-42.3	38
E. euryae	208	4	59	84	-53.8	42
E. feruginae	213	4	64.7	75	-49.6	41
E. japonicum	212	4	53.7	98	-48.9	39
E. pentasporium	157	4	61.8	97	-50.9	39
E. rostrupii	197	4	58.8	81	-50	40
E. vaccinii	200	4	53	94	-49.1	45
E. woronichinii	200	4	53	94	-47.2	40
Query	173	4	58	71	-50.6	48

Table 6: CBC matrix based on the sequence-structure alignment of the 10 species

E. cylindrosporum	0									
E. japonicum	0	0								
E. pentasporium	0	0	0							
E. woronichinii	0	0	0	0						
E. vaccinii	0	0	0	0	0					
E. ferrugineae	0	0	0	0	0	0				
E. rostrupii	1	2	1	1	1	3	0			
E. camelliae	1	0	1	0	0	0	1	0		
E. euryae	2	0	1	0	0	0	1	1	0	
Query	0	0	0	0	0	0	0	0	0	0
	E. cylindrosporum	***E. japonicum***	***E. pentasporium***	***E. woronichinii***	***E. vaccinii***	***E. ferrugineae***	***E. rostrupii***	***E. camelliae***	***E. euryae***	**Query**

B1.4.5. Loop mediated isothermal amplification (LAMP) assay:

Loop mediated isothermal amplification (LAMP) assay is a highly sensitive technique for molecular detection of targeted organisms based on the amplification of DNA. This is used as a low-cost alternative to detect diseases as it is less time-consuming than conventional PCR-based methods and can also be carried out without a thermal cycler. For the lamp assay, the internal transcribed spacer (ITS) region was targeted and primers were designed from the amplified ITS 1150 bp amplicon sequence. The three isolates of *E. vexans* isolated from Happy Valley tea estate (EV_HV_15), Ananda Bagan tea estate (EV_AB_18) and Pathalipam Grant (EV_PG_19) were used in this study. LAMP reaction was performed in a 25µL volume 2 µL of outer primers (F3 and B3), two inner most primers (FIP and BIP), and two loop primers (LoopF and LoopB), 1 mM dNTPs, 4 mM MgCl2, 2.5 µL of 10X BSM Reaction Buffer, 8 U of Bsm DNA polymerase (Thermofisher Scientific) and 1.5µL target DNA (about 100 ng). The reaction was carried out at 62 °C for 60 min in a water bath followed by incubation at 80 °C for 10 min to inactivate the Bsm DNA polymerase. After completing the isothermal amplifcation 1µL of SYBR Green I (Invitrogen, USA) was added for visual assay of the amplification. The change of the reaction mixture to green indicated a positive amplification and when the amplicon was observed in gel electrophoresis positive amplification was seen with the presence of ladder type band. Other major fungal pathogen *viz. Alternaria alternate, Aspergillus flavus, Fusarium solani, Trichoderma viridae, Candida albicans, Colletotrichum gleosporoides* and *Aspergillus Niger* were taken as positive control for the study. From the figure it can be seen that ladder type band and color change to green was observed only for the two strain of *Exobasidium vexans* (Fig.10 a, b). This result indicated that the designed primers from the ITS barcode region for the lamp assay were highly specifc for *E. vexans*, as it can correctly distinguish *E. vexans* amidst the test fungi.

Following the positive LAMP amplification of the *E. vexans* isolates grown *in vitro*, the assay was tested on *E. vexans* gDNA from the infected tea leaves, sourced directly from blister blight infected fields. The gDNA from an uninfected tea leaf, was used as a control for this experiment. As seen in Fig. 10 c, all the three LAMP reactions using the gDNA from the infected leaves could identify the presence of *E. vexans* due to the characteristic ladder-like positive amplification. As expected, no LAMP amplification was observed for the gDNA isolated from the uninfected tea leaf sample. The positive amplification for LAMP reactions were also revalidated with the SYBR green I staining procedure, as discussed earlier, wherein the LAMP assays with infected leaf parts showed colour change (Fig. 10 d).

For determining the sensitivity of the LAMP primers, the *E. vexans* gDNA was diluted to concentrations ranging from 6.01×10^{-1}ng/µl to 6.01×10^{-7}ng/µl and checked for the positive amplification in the LAMP assay. The characteristic ladder-type bands were observed after gel electrophoresis up to a gDNA concentration of 6×10^{-7} ng/µl for all the three isolates (EV_PG_19, EV_AB_18 and EV_HV_15) (Fig. 11 a, b, c). The results were also validated by the visual inspection of colour change wherein the colour change to green was observed in all the three isolates up to 6×10^{-7} ng/µl concentrations (Fig 11 a', b', c'). The LAMP assay was further validated with RealAmp assay. RealAmp assay was performed to obtain the quantitative amplification of the LAMP products, in real-time by tracing the SYBR green fluorescent signal **(Lei et al. 2019)**. The reaction contained 0.8 µM each of FIB and BIP, 0.8 µM each of LF and LB, 0.2 µM each of F3 and B3, 10 mM dNTPs, 2.5 µl 10 × LAMP buffer (KCl, $MgSO_4$, $(NH_4)_2SO_4$, Tris–HCl, TritonX-100), 8 U of the BsmDNA polymerase, 6 ng of the gDNA and 1 µl of SYBR Green dye added prior to amplification. The reaction was carried out for 60 cycles at 62 °C for 30 s (stage 1), 62°C for 15 s (stage 2), and 62 °C for 15 s (stage 3), followed by a melt curve (95 °C for 15 s, 62°C to 80°C, increment 0.5 °C for 0.5 s) **(Cao et al. 2017)**. The fluorescence signals were detected after every step, through the QIAGEN Roto-Genė Q thermal cycler. The sensitivity tests of the LAMP primers for gDNA were also carried out in real-time. Real time amplification curves obtained by detecting the fluorescence signal for positive amplifications were observed for the three isolates of *E. vexans* as shown in Fig. 12 a. As expected, the fluorescence signals of the other test fungi didn't cross the threshold value, indicating a negative amplification for the test fungi (Fig. 12 a,). The sensitivity of the RealAmp assay was carried out to find out the detection limit for the three isolates of *E. vexans* with a 10^{-7}-fold serial dilution of the gDNA. The amplification curve and Ct value for each of the dilution of all the isolates represent positive amplification and indicates a detection limit of up to 6x 10^{-7} ng/µl (Fig12 b, c, d, e). The RealAmp results of the sensitivity test (Fig. 12 b, c, d) also corroborated the gel electrophoresis results of the sensitivity assay shown in Fig. 12 a, b, c.

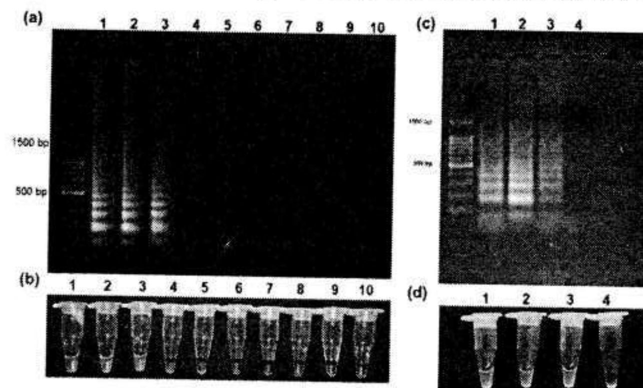


Fig.10. LAMP reaction results visualized with gel electrophoresis (a) and SYBR Green I dye (b) [1. EV_HV_15, (Happy valley) EV_AB_18, (Ananda Bagan) EV_PG_19 (Pathalipam Grant, 3. *Alternaria alternate*, 4. *Aspergillus flavus*, 5. *Aspergillus Niger*, 6. *Candida albicans*, 7. *Colletotrichum gleosporoides*, 8. *Fusarium solani*, 9. *Trichoderma viridae*], Evaluation of Lamp assay for detection of *E. vexans* in infected tea leaf samples after gel electrophoresis (c) and naked eye visualization with SYBR green dye collected from (1) Happy Valley Tea estate (2) Ananda Tea Estate, (3) Pathalipam Grant and (4) Healthy tea leaf part (d).

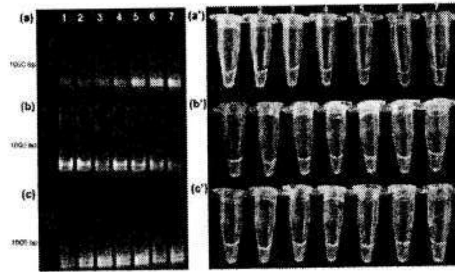
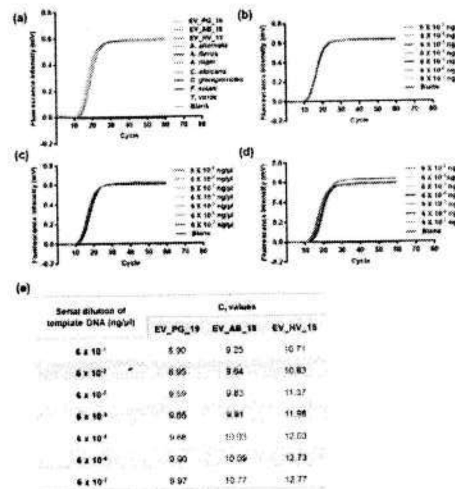


Fig. 11. Sensitivity test for LAMP assay for the detection of *E. vexans*. Gel electrophoresis results showing ladder like band pattern (a, b, c) and naked eye visualization with SYBR green dye (a', b', c') for different concentrations of the three isolates. [(a, a') EV_HV_15, (b, b') EV_AB_18, (c, c') EV_PG_19, (1) 6x 10^{-1} ng/µl, (2) 6x 10^{-2} ng/µl, (3) 6x 10^{-3} ng/µl, (4) 6x 10^{-4} ng/µl, (5) 6x 10^{-5} ng/µl, (6) 6x 10^{-6} ng/µl, (7) 6x 10^{-7} ng/µl].



Serial dilution of template DNA (ng/µl)	C_t values		
	EV_PG_19	EV_AB_18	EV_HV_15
6 x 10^{-1}	8.90	9.25	10.71
6 x 10^{-2}	8.95	9.64	10.83
6 x 10^{-3}	9.59	9.83	11.37
6 x 10^{-4}	9.65	9.91	11.98
6 x 10^{-5}	9.68	10.03	12.03
6 x 10^{-6}	9.90	10.09	12.73
6 x 10^{-7}	9.97	10.77	12.77

Fig. 12. RealAmp amplification assay. Amplification curve obtained from fluorescence detection in real time with amplification for *E. vexans* isolates and test fungi (a), sensitivity test for the detection limit of EV_PG_19 (b), EV_AB_18 (c), EV_HV_15 (d), Ct values for the RealAmp sensitivity assay for *E. vexans* isolates (e).

B1.4.6. Optimization of the growth medium components

Statistical optimization of the growth environment for *Exobasidium vexans* was carried out in order to assess the effect of the growth conditions and the concentration of media components to establish the optimal parameters for in vitro growth. These statistical experimental designs have numerous advantages over conventional optimization procedures and include relatively advanced results with less process variability, closer confirmation, less development time and less overall costs. The optimization of three different media *viz.*

- Potato dextrose broth
- Czapek dox broth
- V8 juice broth

amended with growth supplements *viz.* tea leaf extract and $CaCO_3$ for the growth of mycelia were carried out using the Central Composite Design (CCD) of Response Surface Methodology (RSM). The RSM employed was based on a five-level-five factor central composite design leading to 32 experiments for each growth medium. The boundary parameters for the selected five factors were:

1. Carbon source (g): Initial concentration in media – Double the initial concentration
2. Tea leaf extract (%W/V): 0 - 15 %
3. Initial pH: 5 - 8
4. Temperature (^{0}C): 20 - 30 ^{0}C
5. $CaCO_3$ (g): 0 - 0.8 g

The designed experimental data for each media are depicted below in tabular form. For the calculation of the optimal growth environment, a second order polynomial function was fitted using the following equation to correlate the relationship between the mycelial weight, medium components and growth conditions:

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} b_{ii} X_i^2 + \sum_{i=1}^{n} \sum_{i>j}^{n} b_{ij} X_i X_j$$

Where Y is the predicted response variable, b_0 is the model constant, b_i is the linear co-efficient, b_{ii} is the interaction coefficients, b_{ij} is the quadratic co-efficient and X_i and X_j are the coded values. The Analysis of Variance (ANOVA) for the fitted polynomial model is also shown along with the coefficient of determination, R^2, presenting the quality of fit of the equation. In addition, the graphical representations of the experimental factors having a significant effect on the mycelial growth are presented.

A. Potato Dextrose Broth

Table 7. Central composite design matrix with response of mycelial growth

Experimental Run	Factor 1 Dextrose (g)	Factor 2 Tea Leaf Extract (%W/V)	Factor 3 Initial pH	Factor 4 Temperature (°C)	Factor 5 CaCO3 (g)	Response 1 Mycelial growth (g)
1	0.4	15	6.5	25	0.4	1.44
2	0.3	15	6.5	25	0.8	1.04
3	0.25	22.5	7.25	27.5	0.2	2.5
4	0.3	15	8	25	0.4	1.14
5	0.35	22.5	5.75	22.5	0.6	1.31
6	0.2	15	6.5	25	0.4	0.6
7	0.35	22.5	7.25	22.5	0.2	0.95
8	0.35	7.5	5.75	22.5	0.2	0.81
9	0.3	15	6.5	25	0.4	1.23
10	0.35	22.5	7.25	27.5	0.6	1
11	0.25	22.5	7.25	22.5	0.6	1.06
12	0.35	7.5	5.75	27.5	0.6	1.1
13	0.3	15	5	25	0.4	0.93
14	0.3	0	6.5	25	0.4	0.4
15	0.25	22.5	5.75	22.5	0.2	0.9
16	0.25	7.5	7.25	22.5	0.2	0.64
17	0.3	15	6.5	25	0.4	1.97
18	0.25	7.5	5.75	22.5	0.6	0.9
19	0.25	22.5	5.75	27.5	0.6	1
20	0.3	15	6.5	25	0.4	1.3
21	0.25	7.5	7.25	27.5	0.6	1
22	0.35	7.5	7.25	27.5	0.2	0.9
23	0.3	15	6.5	25	0.4	1.6
24	0.3	15	6.5	25	0.4	1.6
25	0.3	15	6.5	25	0	1.3
26	0.3	15	6.5	25	0.4	1.6
27	0.3	15	6.5	30	0.4	1.1
28	0.35	22.5	5.75	27.5	0.2	1.15
29	0.3	30	6.5	25	0.4	2

30	0.25	7.5	5.75	27.5	0.2	0.7
31	0.3	15	6.5	20	0.4	0.9
32	0.35	7.5	7.25	22.5	0.6	0.8

Table 8. Analysis of variance (ANOVA) for the experimental results of the CCD quadratic model for mycelial growth

Source	Sum of Squares	Degree of freedom	Mean Square	F value	p-value Prob > F	
Model	4.992043	20	0.249602	2.771387	0.0428	Significant
A-Dextrose	0.041667	1	0.041667	0.462634	0.5105	
B-Tea leaf Extract	1.612017	1	1.612017	17.89857	0.0014	Significant
C-Initial pH	0.081667	1	0.081667	0.906763	0.3614	
D-Temperature	0.236017	1	0.236017	2.620544	0.1338	
E-CaCO3	0.03375	1	0.03375	0.374734	0.5529	
AB	0.126025	1	0.126025	1.399283	0.2618	
AC	0.366025	1	0.366025	4.064055	0.0689	
AD	0.126025	1	0.126025	1.399283	0.2618	
AE	0.087025	1	0.087025	0.966258	0.3467	
BC	0.1089	1	0.1089	1.20914	0.295	
BD	0.0484	1	0.0484	0.537396	0.4788	
BE	0.2209	1	0.2209	2.452701	0.1456	
CD	0.2304	1	0.2304	2.558181	0.138	
CE	0.2209	1	0.2209	2.452701	0.1456	
DE	0.2304	1	0.2304	2.558181	0.138	
A^2	0.412855	1	0.412855	4.584014	0.0555	
B^2	0.159055	1	0.159055	1.766017	0.2108	
C^2	0.387167	1	0.387167	4.2988	0.0624	
D^2	0.448388	1	0.448388	4.978548	0.0474	Significant
E^2	0.193105	1	0.193105	2.144082	0.1711	
Residual	0.990704	11	0.090064			
Lack of Fit	0.641904	6	0.106984	1.5336	0.3278	not significant
Pure Error	0.3488	5	0.06976			
Cor Total	5.982747	31				
Std. Dev.= 0.30			Mean = 1.15	Adequate precision = 8.028		
	$R^2 = 0.83$				Adj. $R^2 = 0.73$	

The second-order polynomial model for the mycelial growth in terms of coded factors is based on the following equation:

Mycelial growth = - 31.88396 + 0.73873 x Dextrose + 7.49495 – 003 x Tea leaf Extract + 2.43626 x Initial pH + 0.87776 x Temperature + 11.48939 x CaCO3 - 2.36667 - 003 x Dextrose x Tea leaf Extract - 0.040333 x Dextrose x Initial pH - 7.10000 - 003 x Dextrose x Temperature + 0.073750 x Dextrose x $CaCO_3$ + 0.014667 x Tea leaf Extract x Initial pH + 2.93333 - 003 x Tea leaf Extract x Temperature - 0.078333 x Tea leaf Extract x $CaCO_3$ + 0.064000 x Initial pH x Temperature - 0.78333 x Initial pH x $CaCO_3$ - 0.24000 x Temperature x $CaCO_3$ - 4.74545 - 003 x $Dextrose^2$ - 1.30909 - 003 x Tea leaf $Extract^2$ - 0.20424 x Initial pH^2 - 0.019782 x $Temperature^2$ - 2.02841 x $CaCO_3^2$

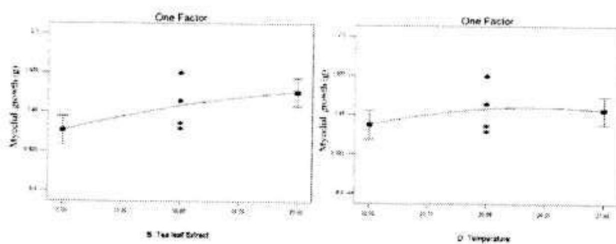


Fig. 13. Uni-parametric response curve of mycelium biomass of *Exobasidium vexans* grown on Potato Dextrose broth medium

B. Czapek Dox Broth

Table 9. Central composite design matrix with response of mycelial growth

Experimental Run	Factor 1 Sucrose (g)	Factor 2 Tea Leaf Extract (%W/V)	Factor 3 Initial pH	Factor 4 Temperature (°C)	Factor 5 CaCO3 (g)	Response 1 Mycelial growth (g)
1	0.6	15	6.5	25	0.4	5.8
2	0.45	0	6.5	25	0.4	3.22
3	0.375	22.5	7.25	27.5	0.2	2
4	0.045	15	5	25	0.4	1.45
5	0.525	7.5	5.75	22.5	0.2	3.5
6	0.525	7.5	7.25	27.5	0.2	1.6
7	0.525	22.5	5.75	22.5	0.6	2.92
8	0.525	7.5	7.25	22.5	0.6	4.2
9	0.045	15	6.5	25	0.4	1.5
10	0.375	7.5	5.75	22.5	0.6	1.66
11	0.045	15	6.5	25	0	1.4
12	0.525	22.5	7.25	22.5	0.2	2.78
13	0.525	7.5	5.75	27.5	0.6	1.9
14	0.375	22.5	5.75	27.5	0.6	2
15	0.45	15	6.5	25	0.4	1.5
16	0.525	22.5	7.25	27.5	0.6	4.8
17	0.45	15	6.5	30	0.4	2.1
18	0.45	15	6.5	25	0.4	1.7
19	0.525	22.5	5.75	27.5	0.2	5.4
20	0.375	7.5	7.25	27.5	0.6	4.5
21	0.3	15	6.5	25	0.4	3.5
22	0.375	7.5	5.75	27.5	0.2	4
23	0.45	15	6.5	25	0.8	2.1
24	0.45	30	6.5	25	0.4	1.8
25	0.375	22.5	5.75	22.5	0.2	1.5

26	0.45	15	8	25	0.4	1.93
27	0.45	15	6.5	25	0.4	1.2
28	0.375	22.5	7.25	22.5	0.6	1.58
29	0.375	7.5	7.25	22.5	0.2	2.34
30	0.45	15	6.5	25	0.4	1.1
31	0.45	15	6.5	25	0.4	1.2
32	0.45	15	6.5	20	0.4	2.3

Table 10. Analysis of variance (ANOVA) for the experimental results of the CCD quadratic model for mycelial growth

Source	Sum of Squares	Degree of freedom	Mean Square	F value	p-value Prob > F	
Model	50.32856	20	2.516428	14.70468	< 0.0001	Significant
A-Sucrose	6.1206	1	6.1206	35.76556	< 0.0001	Significant
B- Tea leaf extract	0.528067	1	0.528067	3.085743	0.1067	
C-Initial pH	0.147267	1	0.147267	0.860549	0.3735	
D-Temperature	1.179267	1	1.179267	6.891012	0.0236	Significant
E-CaCO3	0.141067	1	0.141067	0.824319	0.3834	
AB	6.4009	1	6.4009	37.40348	< 0.0001	Significant
AC	0.16	1	0.16	0.934956	0.3544	
AD	1.6384	1	1.6384	9.573945	0.0102	Significant
AE	0.0256	1	0.0256	0.149593	0.7063	
BC	0.3136	1	0.3136	1.832513	0.203	
BD	1.6384	1	1.6384	9.573945	0.0102	Significant
BE	0.09	1	0.09	0.525912	0.4835	
CD	0.1849	1	0.1849	1.080458	0.3209	
CE	9.4249	1	9.4249	55.07414	< 0.0001	Significant
DE	0.0001	1	0.0001	0.000584	0.9811	
A^2	20.28527	1	20.28527	118.5364	< 0.0001	Significant
B^2	2.580341	1	2.580341	15.07815	0.0025	Significant
C^2	0.246074	1	0.246074	1.437928	0.2557	
D^2	1.408024	1	1.408024	8.227751	0.0153	Significant
E^2	0.333274	1	0.333274	1.947479	0.1904	
Residual	1.882442	11	0.171131			
Lack of Fit	1.609109	6	0.268185	4.90582	0.0509	Not significant
Pure Error	0.273333	5	0.054667			
Cor Total	52.211	31				
Std. Dev.= 0.41			Mean = 2.51		Adequate precision= 12.94	
	$R^2 = 0.96$				Adj. $R^2 = 0.89$	

The second-order polynomial model for the mycelial growth in terms of coded factors is based on the following equation:

Mycelial growth = + 47.19227 - 0.90032 x Sucrose - 0.92885 x Plant leaf extract - 1.45232 x Initial pH - 0.77739 x Temperature - 35.33182 x $CaCO_3$ + 0.011244 x Sucrose x Plant leaf extract - 0.017778 x Sucrose x Initial pH - 0.017067 x Sucrose x Temperature + 0.026667 x Sucrose x $CaCO_3$ - 0.024889 x Plant leaf extract x Initial pH + 0.017067 x Plant leaf extract x Temperature - 0.050000 x Plant leaf extract x $CaCO_3$ - 0.057333 x Initial pH x Temperature + 5.1166 x Initial pH x $CaCO_3$-5.00000E-003 x Temperature x $CaCO_3$ + 0.014784 x $Sucrose^2$ + 5.27273 - 003 x Plant leaf $extract^2$ + 0.16283 x Initial pH^2 + 0.035055 x $Temperature^2$ + 2.66477 x $CaCO_3{}^2$

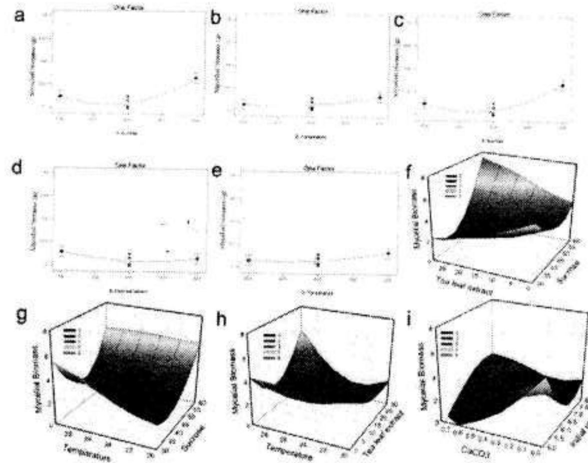


Fig. 14. Uni-parametric (a-e) and bi-parametric (f-i) response curve of mycelium biomass of *Exobasidium vexans* grown on Czapek Dox broth medium

C. **V8 Juice Broth**

Table 11. Central composite design matrix with response of mycelial growth

Experimental Run	Factor 1 Glucose (g)	Factor 2 Tea Leaf Extract (%W/V)	Factor 3 Initial pH	Factor 4 Temperature (°C)	Factor 5 CaCO3 (g)	Response 1 Mycelial growth (g)
1	0.03	15	6.5	25	0.5	1.55
2	0.035	7.5	5.75	22.5	0.35	1.98
3	0.03	15	6.5	25	0.5	1.68
4	0.025	22.5	5.75	27.5	0.65	1.96
5	0.025	22.5	7.25	22.5	0.65	1.32
6	0.025	7.5	7.25	22.5	0.35	0.84
7	0.03	15	6.5	25	0.5	1.55
8	0.025	7.5	5.75	27.5	0.35	1.7
9	0.02	15	6.5	25	0.5	1.27
10	0.03	15	6.5	20	0.5	1.42
11	0.03	15	6.5	25	0.5	1.1
12	0.025	22.5	5.75	22.5	0.35	1.43
13	0.035	22.5	7.25	22.5	0.35	1.45

14	0.035	22.5	5.75	27.5	0.35	1.61
15	0.04	15	6.5	25	0.5	2.35
16	0.03	30	6.5	25	0.5	1.8
17	0.03	15	6.5	25	0.2	0.96
18	0.03	0	6.5	25	0.5	1.52
19	0.035	7.5	7.25	27.5	0.35	0.76
20	0.035	22.5	7.25	27.5	0.65	2.44
21	0.035	22.5	5.75	22.5	0.65	1.49
22	0.025	7.5	7.25	27.5	0.65	1.43
23	0.035	7.5	7.25	22.5	0.65	1.4
24	0.03	15	6.5	25	0.5	1.55
25.	0.03	15	6.5	30	0.5	1.62
26	0.03	15	5	25	0.5	1.37
27	0.03	15	8	25	0.5	1.27
28	0.03	15	6.5	25	0.8	1.35
29	0.035	7.5	5.75	27.5	0.65	3.22
30	0.03	15	6.5	25	0.5	1.55
31	0.025	7.5	5.75	22.5	0.65	1.14
32	0.025	22.5	7.25	27.5	0.35	0.7

Table 12. Analysis of variance (ANOVA) for the experimental results of the CCD two- factor interaction model for mycelial growth

Source	Sum of Squares	Degree of freedom	Mean Square	F value	p-value Prob > F	
Model	6.288217	15	0.419214	5.122637	0.0012	significant
A-Glucose	1.495004	1	1.495004	18.26837	0.0006	significant
B-Tea leaf Extract	0.010004	1	0.010004	0.122247	0.7312	
C-Initial pH	0.803004	1	0.803004	9.812397	0.0064	significant
D-Temperature	0.418704	1	0.418704	5.116401	0.038	
E-CaCo3	0.924338	1	0.924338	11.29504	0.004	significant
AB	0.028056	1	0.028056	0.342836	0.5664	
AC	0.006006	1	0.006006	0.073394	0.7899	
AD	0.026406	1	0.026406	0.322674	0.5779	
AE	0.154056	1	0.154056	1.882507	0.189	
BC	0.573806	1	0.573806	7.011688	0.0175	significant
BD	0.033306	1	0.033306	0.406989	0.5325	
BE	0.000756	1	0.000756	0.009241	0.9246	
CD	0.283556	1	0.283556	3.464947	0.0812	
CE	0.191406	1	0.191406	2.33891	0.1457	
DE	1.339806	1	1.339806	16.37191	0.0009	
Residual	1.309371	16	0.081836			
Lack of Fit	1.107038	11	0.10064	2.48698	0.1624	not significant
Pure Error	0.202333	5	0.040467			

Cor Total	7.597588	31		
Std. Dev.= 0.29		Mean = 1.52		Adequate precision = 11.045
	$R^2 = 0.82$			Adj. $R^2 = 0.76$

The second-order polynomial model for the mycelial growth in terms of coded factors is based on the following equation:

Mycelial growth = + 6.17021 - 0.46417 x Glucose - 0.12483 x Tea leaf Extract + 0.69500 x Initial pH + 0.067500 x Temperature - 28.31944 x $CaCO_3$ - 0.011167 x Glucose x Tea leaf Extract - 0.051667 x Glucose x Initial pH x 0.032500 x Glucose xTemperature + 1.30833 x Glucose x $CaCO_3$ + 0.033667 x Tea leaf Extract x Initial pH - 2.43333 - 003 x Tea leaf Extract x Temperature + 6.11111 – 003 x Tea leaf Extract x $CaCO_3$ - 0.071000 x Initial pH x Temperature + 0.97222 x Initial pH x $CaCO_3$ + 0.77167 x Temperature x $CaCO_3$

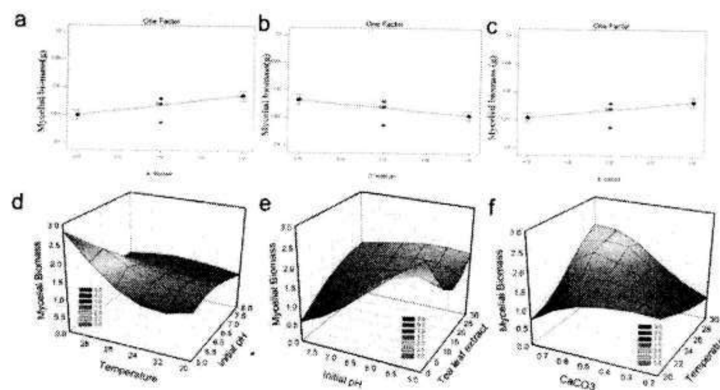


Fig. 15. Uni-parametric (a-c) and bi-parametric (d-f) response curve of mycelium biomass of *Exobasidium vexans* grown on V8 Juice broth medium

Establishment of the optimal growth condition for the three media was successfully mediated using RSM methodology. The close proximity between R^2 and Adj. R^2 in ANOVA for the three models confirmed the consistency of the fitted model. For PDB media, the optimal growth condition was found to be 0.25g of carbon source, 22.5% W/V tea leaf extract, 7.25 pH, 27.5 °C temperature and 0.2g of $CaCO_3$ in 100ml of H_2O. For CDB media the optimal growth condition was 0.6g of carbon source, 15% W/V tea leaf extract, 6.5 pH, 25 °C temperature and 0.4g of $CaCO_3$ for 100ml of H_2O. For V8 juice broth the optimal growth condition was 0.035g of carbon source, 7.5% W/V tea leaf extract, 5.75 pH, 27.5 °C temperature and 0.65g of $CaCO_3$ for 100ml of H_2O. The results suggest that czapek dox media is the optimal media for the growth of *Exobasidium vexans*. Further, tea leaf extract was found to play a significant role for the in vitro growth of *Exobasidium vexans*.

B1.4.7. Draft genome sequence of *E. vexans* and pathogenic mechanism

DNA was isolated from given sample by Xcelgen Bacterial DNA isolation Kit. Quality of DNA was checked on 0.8% agarose gel (loaded 3 µl) for the single intact band. The paired-end sequencing library was prepared using NEBNext Ultra DNA Library Prep Kit for Illumina. The amplified library was analyzed in Bioanalyzer 2100 (Agilent Technologies) using High Sensitivity (HS) DNA chip as per manufacturer's instructions. The average size of library is 354bp respectively for given sample.The library was sequenced on Illumina platform (2 x 150bp chemistry) to generate ~6 GB data.

Table. 13. Details of software used during the assembly and analysis of *E. vexans* genome

Software	Application
SoapDenovo	Denovo assembly of reads

Gap closer	Gap filling in assembled scaffolds
SSPACE	scaffolding
tRNAscan-SE	tRNAs identification
rnnamer	rRNAs identification
Genemark-ES	Genes prediction
Blastp	Similarity search against NR
Blast2GO	Go mapping and annotation
KAAS	Pathway Analysis against KEGG database
Misa.pl	SSR identification
PHI database	Pathogenesis related genes identification
CAZyme database	Identification of carbohydrate machinery

The genome of *E. vexans* was sequenced on Illumina platform by extracting good quality gDNA. A total of 47,613,220 raw reads and 7,062,461,452 bases were generated by paired-end sequencing. The reads were integrated with the *de novo* assembly of Illumina sequences with SOAP denovo software and the final assembly resulted into 318 number of scaffolds. The %GC content of the draft genome *E. vexans* was 48.11%. The predicted genes were with 30780 bp gene length and mean gene size of 1437. The functional annotation of genes was performed with KEGG orthology and is shown in Fig. 16.

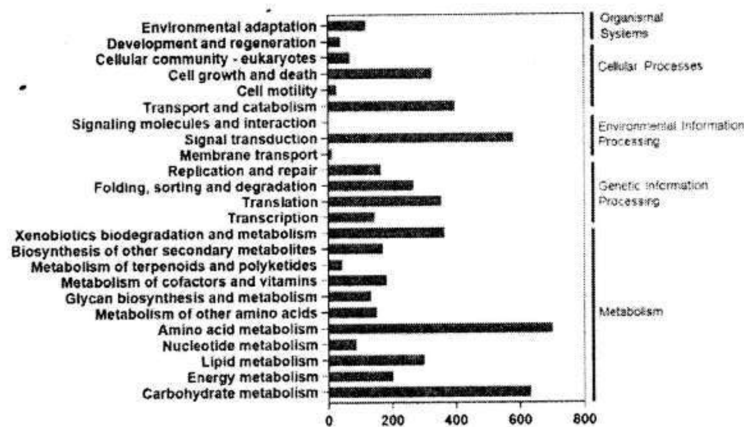


Fig. 16. Functional annotations of predicted *E. vexans* proteins based on KEGG Orthology

Gene families involved in pathogenesis

Potential genes in *E. vexans* involved in pathogenicity was analyzed by performing BLAST using the protein sequences in the Pathogen-Host Interaction Database (PHI database) (Fig. 17). A total of 1697 protein coding genes in E. vexans were predicted to be orthologous to PHI genes. Out of these genes 138 genes were found to be pathogenicity factors as mutant of these genes showed loss of pathogenicity. 650 genes were putatively found to regulate virulence and 06 effector genes were found homologous to PHI database which were plant avirulence determinant gene.

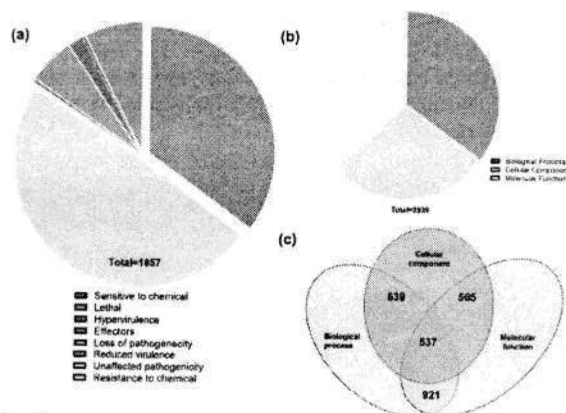


Fig. 17. Classification of the *E. vexans* secretome based on the Pathogen-Host Interaction database (PHI-base)

B1.4.8. Development of antifungal nanoparticles for the effective management of blister blight infection on tea caused by *Exobasidium vexans*

Nanocellulose:Cu:ZnS nanocomposite were synthesized through a modified solvothermal approach (**Wu et al. 2012**) using two different capping agents: Mercaptopropionic acid (MPA) and Sodium citrate (SC). Stoichiometric amounts of $ZnCl_2$ (8 mM), Na_2SO_3 (50 mM) and $Cu(CH_3COO)_2$ (8 mM) were used to obtain the solvothermal premix. Rice husk was used as a cellulosic precursor for the synthesis of the nanocomposites. The two capping agents, viz. SC (19 mM) and MPA (19 mM) was added separately into the premix, in separate reaction setups. The as prepared reaction mixture was then stirred on a magnetic stirrer for a period of 1 h with gradual addition of $NaBH_4$ (75 mM), as the reducing agent. It was then transferred into a round bottom flask and was subjected to solvothermal treatment at a temperature of 80° C for different reflux intervals viz. 1–5 h to generate the time-based variants. The solvothermal extract was cooled to room temperature, followed by washing with copious amounts of de-ionised water and ethanol to remove all unreacted precursors. The washed nanosystems were finally oven dried at a temperature of 60°C for a period of 48 h.

Characterization of the synthesized antifungal nanoparticles

The as prepared nanosystems were characterized using Scanning Electron Microscopy (SEM), SEM EDX, powder X-ray Diffraction (XRD) spectroscopy and Fourier Transform Infrared spectroscopy (FTIR) for the investigation of their structural and functional attributes. The average size of the nanosystems was estimated using Photon Correlation Spectroscopy and the electrostatic stability of the nanosystems was assessed via surface charge (ζ potential) analysis.

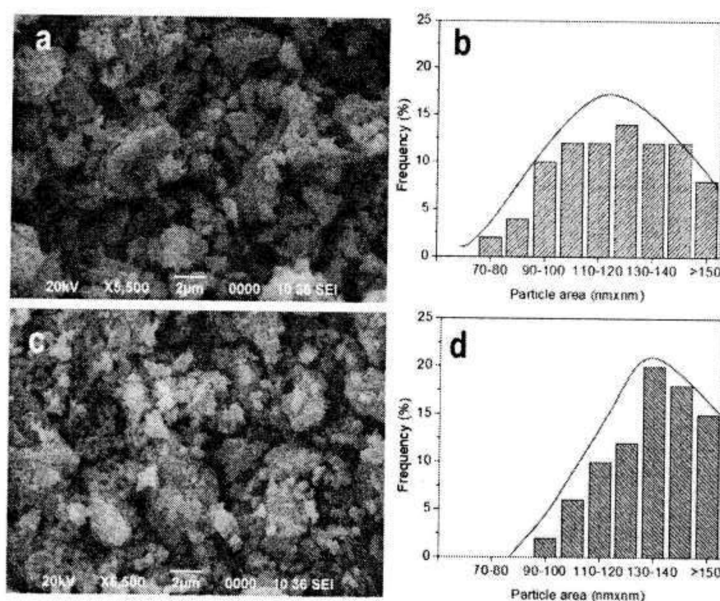


Fig. 18. SEM micrograph and particle size distribution of 1 h variant of (a, b) SC@NC:Cu:ZnS and (c, d) MPA@NC:Cu:ZnS

Fig. 18 a, c and b, d shows the SEM micrographs and histogram depicting size distribution of the SC@NC:Cu:ZnS and MPA@NC:Cu:ZnS for the 1 h variant. The synthesized MPA@NC:Cu:ZnS and SC@NC:Cu:ZnS nanosystems are seen to exhibit a quasi-

spherical microstructure. From the particle size distribution for the NC:Cu:ZnS nanosystems the MPA@NC:Cu:ZnS nanosystems was found to have a narrower size distribution compared to the SC@NC:Cu:ZnS nanosystems. Fig. 19 depicts the pseudo-colour elemental maps for C, O, S, Cu and Zn of the SC@NC:Cu:ZnS and MPA@NC:Cu:ZnS nanosystems that shows a homogenous distribution of the elements throughout the area being investigated.The elemental composition obtained from SEM-EDX studies confirmed the existence of C, O, S, Cu and Zn as elemental constituents in both the functionalized nanosystems (Table 14).

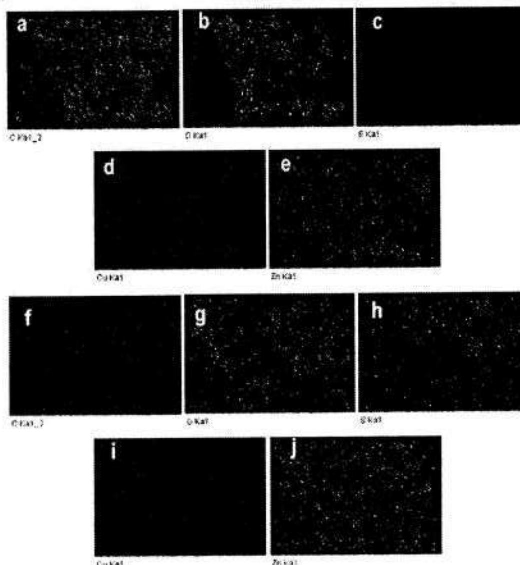


Fig. 19. Pseudo-colour elemental distribution maps of SC@NC:Cu:ZnS (a–e) and MPA@NC:Cu:ZnS (f-j)

Table 14. Elemental composition of the synthesized nanosystem

Element	Weight %		Atomic %	
	SC@NC:Cu:ZnS	MPA@NC:Cu:ZnS	SC@NC:Cu:ZnS	MPA@NC:Cu:ZnS
C K	34.4	5.01	47.37	15.56
O K	45.09	15.82	46.61	36.88
S K	2.74	2.65	1.41	3.08
Cu K	13.87	51.4	3.61	30.16
Zn K	3.9	25	0.99	14.33
Total	100	100		

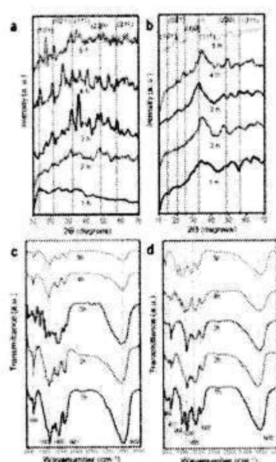


Fig. 20. XRD spectra of (a) SC@NC:Cu:ZnS (b) MPA@NC:Cu:ZnS (b) and FTIR spectra of (c) SC@NC:Cu:ZnS (d) MPA@NC:Cu:ZnS

Fig. 20 a, b depicts the X-ray diffractogram of the SC@NC:Cu:ZnS and MPA@NC:Cu:ZnS nanocomposites for all the reflux variants respectively. Both the diffractograms show the presence of characteristic peaks for the cellulose I crystalline structure of cellulose at 2Θ angles 15.1°, 22.4° corresponding to the diffraction planes 101 and 021 respectively (**Niu et al. 2017**). An additional characteristic peak for cellulose crystalline structure was observed for MPA@NC:Cu:ZnS at 2Θ angles 25° corresponding to diffraction plane 002. Additionally, characteristic peak for zinc blende phase structure of ZnS was observed at 2Θ angles 32°, 48°, 57°, corresponding to the diffraction planes 111, 220 and 311 (**Chauhan et al. 2014**) respectively for both the nanocomposites. The radius of ionic Cu (0.057 nm) has been reported to be congruent to that of Zn (0.06 nm) and hence no separate crystallinity peak for Cu was observed in the diffractograms of the synthesized nanosystems (**Lee et al. 2014**).

The Fourier Transform Infrared (FTIR) spectra of both the nanocomposites are analyzed in the range of 500-4000 cm^{-1} (Fig. 20 c, d). The FTIR spectra of SC@NC:Cu:ZnS (Fig. 20 c) showed dominant infrared signature at 600 cm^{-1} and 1118 cm^{-1} which corresponds to the ZnS vibration and stretching vibration of C-O-C linkage in the glycosidic rings of cellulose respectively (**Kuppayee et al. 2011, Niu et al. 2017**). Additional characteristic peak at 3432 cm^{-1} can be attributed to OH vibration of the O_3: H-O_5 intramolecular hydrogen bond of cellulose reflecting hydrophilic tendency of cellulose (**Li and Renneckar 2011**). The infrared signature at 1400 cm^{-1} corresponds to aromatic skeletal vibration of phenolic group of lignin (**Yang et al. 2007**). The characteristic signature for the presence of capping agent SC is attributed to the peak at 1601 cm^{-1} which corresponds to the stretching vibration of citrate group (**Reghuram et al. 2015**). The FTIR spectra of MPA@ NC:Cu:ZnS (Fig. 20 d) show prominent Zn–S vibration peaks at 485 cm^{-1} and 617 cm^{-1} (**Kuppayee et al. 2011**) along with characteristic peak at 983 cm^{-1} which can be attributed to the resonance interaction between Zn and Cu. This is an indicative of the incorporation of Cu as Cu–S–Zn in the nanosystems (**Lee et al. 2014**). The additional peaks at 1135 cm^{-1} and 3432 cm^{-1} corresponds to C-O stretching vibration and OH stretching vibration of cellulose respectively (**Pan et al. 2016**). Moreover, the infrared signature at 1400 cm^{-1} corresponds to aromatic skeletal vibration of phenolic group of lignin (**Yang et al. 2007**). The peak at 1667 cm^{-1} represents the stretching vibration of thiol group thus suggesting the confirmation of capping of the nanosystems by MPA (**Cheng et al. 2009**).

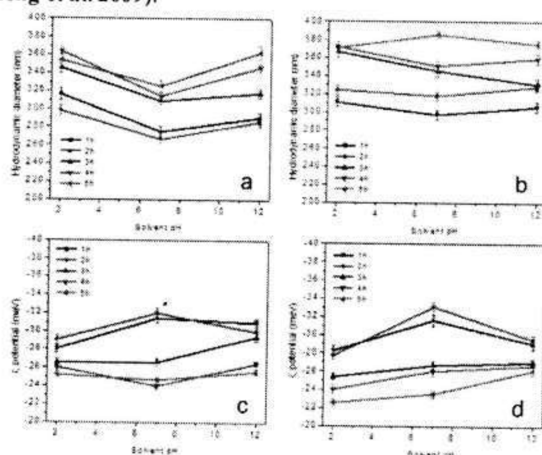


Fig. 21. Hydrodynamic diameter of SC@NC:Cu:ZnS (a) and MPA@NC:Cu:ZnS (b) and ζ potential of SC@NC:Cu:ZnS (c) and MPA@NC:Cu:ZnS (d)

The variation in the hydrodynamic diameter (Fig. 21 a, b) and electrostatic stability (Fig. 21 c, d) of SC@NC:Cu:ZnS and MPA@NC:Cu:ZnS nanocomposites at different solvent pH is shown in fig. 21. From the figure it can be observed that the hydrodynamic diameter of the different variants of the nanocomposites does not show any significant change in relation to their solvent pH. Additionally, the size of the synthesized SC@NC:Cu:ZnS nanocomposites is slightly smaller in comparison to MPA@NC:Cu:ZnS nanocomposites. It has been reported that colloidal dispersions having a ζ potential of ≥ ±20 meV are generally considered to be stable. The ζ potential of both the nanocomposites is found to be in the range of -22 meV to -34 meV (**Ardani et al. 2017**) wherein the 2-hour reflux variant of both the nanocomposites is found to be highly stable with maximum ζ potential.

Antifungal assay using disk diffusion method and broth dilution method

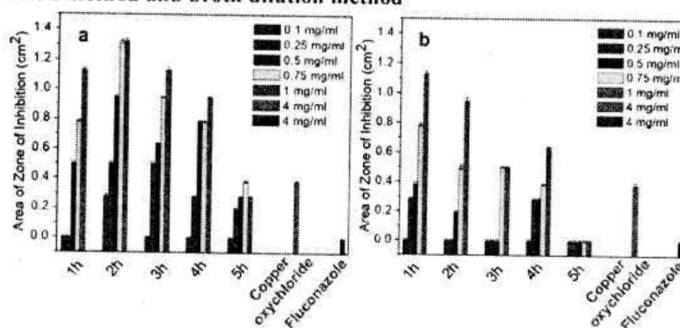


Fig. 22. Antifungal properties of (a) SC@NC:Cu:ZnS (b) MPA@NC:Cu:ZnS against *E. vexans*

Table 15. Minimum inhibitory concentration (MIC_{50}) for the synthesized nanocomposite studied from broth dilution method

Nanocomposite variant	MIC_{50} ($mgml^{-1}$)	
	SC@NC:Cu:ZnS	MPA@NC:Cu:ZnS
1h	0.1	0.25
2h	0.1	0.25
3h	0.1	0.25
4h	0.1	0.5
5h	0.5	1

Fig. 22 shows the comparative analysis of the antifungal efficacy of the two differentially capped NC:Cu:ZnS nanocomposites against *E. vexans*. From the bar diagram, SC@NC:Cu:ZnS was found to be more efficient as compared to MPA@NC:Cu:ZnS with maximum zone of inhibition ~1.4 cm^2 for the 2h variant. For the MPA@NC:Cu:ZnS maximum zone of inhibition was found for the 1h variant. The antifungal efficacy of both the nanocomposite were compared to two commonly used chemical fungicide namely Copper oxychloride (COC) and Fluconazole. From disk diffusion assay it was found that both the nanocomposites showed appreciable antifungal efficiency as compared to COC as prominent zone of inhibition was observed for COC at 4 mg/ml concentration which is much higher than the concentration used for the synthesized nanocomposites. Also, for fluconazole no zone of inhibition was found upto 4mg/ml concentration. Table 15 shows the minimum inhibitory concentration wherein 50% of fungal *invitro* growth was inhibited.

B3. Details of New Leads Obtained, if any:

The salient findings are as follows:

- Isolation of *Exobasidium vexans* strain from the diseased part of the blister blight infected leaves was carried out followed by the identification of the fungus using microscopic technique. Single spore culture, further confirmed the establishment of pure culture of *E. vexans*. *In vitro* germination of basidiospore study revealed that the germination i.e. the formation of germ tube started 6h post incubation and formation of hyphal branching and anastomosis took place at 16h post incubation.
- ITS DNA barcode was developed using designed primers for the species-specific identification of *E. vexans* using MP, ML and BI phylogeny. Divergence time estimation and RNA secondary structure analysis for ITS2 region of *E. vexans* was studied to understand the phylogenetic and evolutionary relationship among other species of Exobasidium genus.
- A loop-mediated isothermal amplification method was developed for the rapid diagnosis of blister blight infection with *E. vexans*, in tea plantations.
- Establishment of the optimal growth condition for *E. vexans* in three media was successfully mediated using RSM methodology. For PDB media, the optimal growth condition was found to be 0.25g of carbon source, 22.5% W/V tea leaf extract, 7.25 pH, 27.5 ^{0}C temperature and 0.2g of $CaCO_3$ in 100ml of H2O. For CDB media the optimal growth condition was 0.6g of carbon source, 15% W/V tea leaf extract, 6.5 pH, 25 ^{0}C temperature and 0.4g of $CaCO_3$ for 100ml of H2O. For V8 juice broth the optimal growth condition was 0.035g of carbon source, 7.5% W/V tea leaf extract, 5.75 pH, 27.5 ^{0}C temperature and 0.65g of $CaCO_3$ for 100ml of H_2O. The results suggest that czapek dox media is the optimal media for the growth of *E. vexans*. Further, tea leaf extract was found to play a significant role for the in vitro growth of *E. vexans*.
- The *in vitro* infection studies were carried out, that validated the virulence potency of the established *in vitro* culture. The in vitro infection studies also mediated the understanding of the pathogenesis of E. vexans, where in it was found that the infection on tea leaf started 3dpi, as evident from the extensive fungal colonization and the formation of fungal appressorium at this stage. Also, in *in vitro* infection assay with tomato seedlings leaf curling was evident from 3dpi. The *in vitro* infection assay in tomato seedlings mediated the establishment of alternate host for *E. vexans* which can be further used as disease model to study pathogenesis of *E. vexans* under laboratory condition.
- Draft genome of *E. vexans* was assembled and analysis of the genome was carried out that provided insights into pathogenic mechansim.
- SC@NC:Cu:ZnS and MPA@NC:Cu:ZnS nanocomposites were synthesized that showed antifungal efficacy against *Exobasidium vexans* strain and performed better when compared to commercially available fungicides.

B4. Details of Publications & Patents, if any:

Manuscript published

- Chaliha, C., Kalita, E., & Verma, P. K. (2020). Optimizing *In vitro* Culture Conditions for the Biotrophic Fungi Exobasidium vexans Through Response Surface Methodology. *Indian Journal of Microbiology*, 60, pages167–174.
- Chaliha, C., Nath, B. K., Verma, P. K., & Kalita, E. (2019). Synthesis of functionalized Cu: ZnS nanosystems and its antibacterial potential. *Arabian Journal of Chemistry*, *12*(4), 515-524.
- Chaliha, C., Rugen, M. D., Field, R. A., & Kalita, E. (2018). Glycans as modulators of plant defense against filamentous pathogens. *Frontiers in plant science*, *9*, 928.

Manuscript under communication

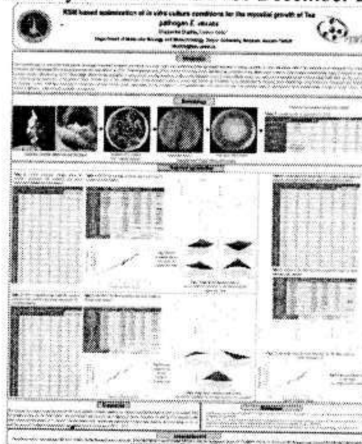
- Loop mediated isothermal amplification (LAMP) assay for rapid, precise and field-deployable identification of tea pathogen *Exobasidium vexans*. Journal: Scientific Reports (under revision)

- Bipartite molecular approach for species delimitation and resolving cryptic speciation of *Exobasidium vexans* within the *Exobasidium* genus. Journal: Computational Biology and Chemistry. (Under revision)

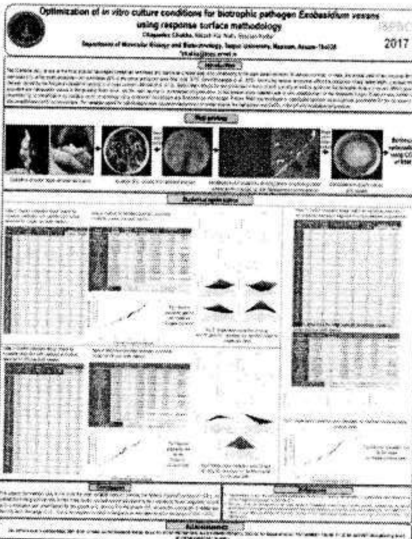
B5. Conference Publications:

- Bipartite molecular approach for species delimitation and resolving cryptic speciation of *Exobasidium vexans* within the *Exobasidium* genus. Chayanika Chaliha[a], V. Chandra Kaladhar, Robin Doley, Praveen Kumar Verma, Aditya Kumar & Eeshan Kalita. Oral presentation at The 19th International Conference on Bioinformatics 2020 held virtually from Nov 25-29, 2020

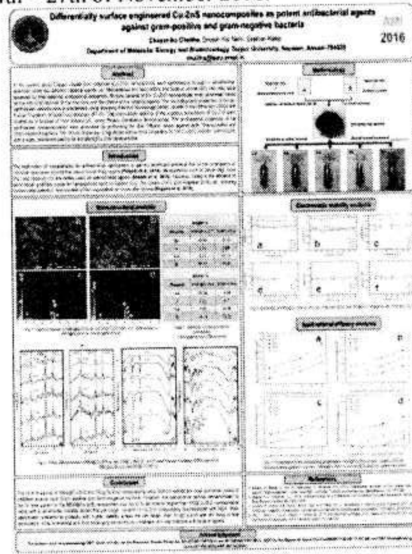
- RSM based optimization of *in vitro* culture conditions for the mycelial growth of Tea pathogen *E. vexans* Chaliha, C., Nath B.K. & Kalita, E., at the "Organix 2020" held at Tezpur University on the 20th -21st of December 2018



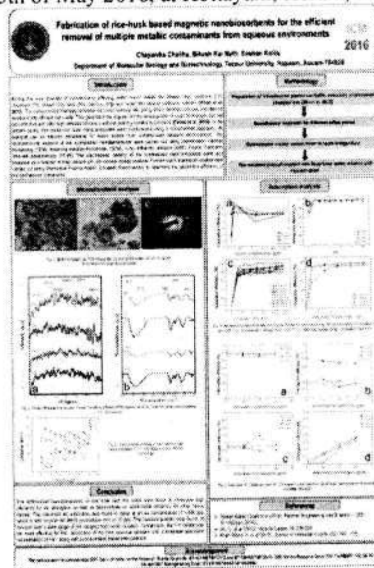
- *Optimization of in vitro culture conditions for biotrophic pathogen Exobasidium vexans using response surface methodology.* Chaliha, C., Nath B.K. & Kalita, E., at the "International Symposium on Plant Biotechnology and Crop Improvement-2017 (ISPBCI-2017)" held at IIT-Guwahati on the 20th -21st of January 2017



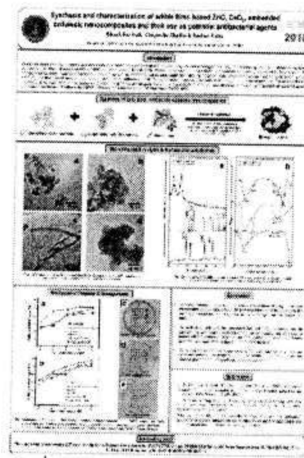
- *Differentially surface engineered Cu:ZnS nanocomposites as potent antibacterial agents against gram-positive and gram-negative bacteria.* Chaliha, C., Nath B.K. & Kalita, E., at the "57th International Annual Conference of The Association of Microbiologists of India (AMI-2016)" held at the Gauhati University, on the 24th – 27th of November 2016.



- *Antibacterial activity of differentially surface engineered Cu:ZnS nanocomposites:Synthesis and characterization*. Chaliha, C., Nath B.K. & Kalita, E., at the "International Conference on Macromolecules, Synthesis, Morphology, Processing, Structure, Properties and Applications", at Mahatma Gandhi University, on the 13th – 15th of May 2016, at Kottayam, Kerala, India.



- *Synthesis and characterization of edible films based ZnO, CeO2, embedded cellulosic nanocomposites and their use as potential antibacterial agents*. Nath B.K., Chaliha, C. & Kalita, E., at the "International Conference on Macromolecules: Synthesis, Morphology, Processing, Structure, Properties and Applications", at Mahatma Gandhi University, on the 13th – 15th of May 2016, at Kottayam, Kerala, India.



B6. Manuscript under preparation:

- Lignocellulose based Cu:ZnS antifungal nanocomposites against blister blight pathogen *Exobasidium vexans*
- Draft genome of *Exobasidium vexans* and insights into pathogenic mechanism

References:

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(Signature of Principal Investigator)
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Assistant Professor,
Dept. of Molecular Biology & Biotechnology,
Tezpur University,
Napaam, Assam, 784028
ekalita@tezu.ernet.in, eeshankalita@gmail.com

Date: 07.01.2020
Place: Tezpur

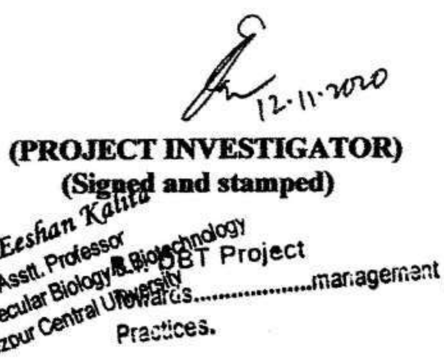
No. ....
Dt. of Receipt ....
Received by ....

Assets acquired wholly or substantially out of Government grants
Register to be maintained by Grantee Institution

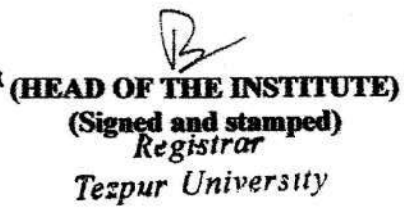
Name of the Sanctioning Authority: **Department of Biotechnology, Govt. of India**

1. Sl. No.: **Agri/2013/145**
2. Name of Grantee Institution: **Tezpur University**
3. No. & Date of sanction order: **BT/427/NE/TBP/2013 dated 25.03.2015**
4. Amount of the sanctioned grant: **₹ 19,66,000.00 (Non-recurring head)**
5. Brief purpose of the grant: For the purchase of equipments and accessories
6. Whether any condition regarding the right of ownership of Govt. in the property or other assets acquired out of the grant was incorporated in the grant –in-aid sanction order.: Not Applicable
7. Particulars of assets actually credited or acquired.: **Please refer Appendix A1**
8. Value of the assets as on 30.06.20120: **₹ 19,88,256.00**
9. Purpose for which utilized at present: Not Applicable
10. Encumbered or not: Not Applicable
11. Reasons, if encumbered: Not Applicable
12. Disposed of or not: Not Applicable
13. Reasons and authority, if any, for disposal: Not Applicable
14. Amount realized on disposal: Not Applicable
15. Remarks: A total amount of ₹1038836 via wire transfer from equipment head paid as advance in the financial year 2017-2018 for equipment has been re-adjusted in financial year 2020-2021 from equipment head (₹1020084) and Overhead (₹18752). An amount of ₹18752 had to be readjusted from overhead grant due to change in currency exchange rate.

(FINANCE OFFICER)
(Signed and stamped)
Finance Officer
Tezpur University



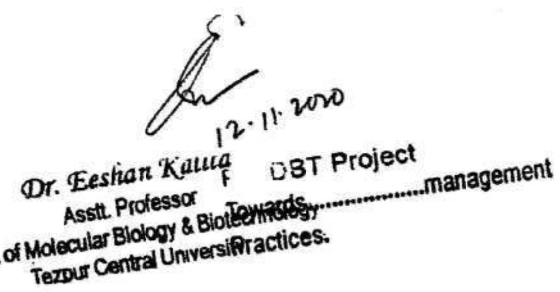
(PROJECT INVESTIGATOR)
(Signed and stamped)
Dr. Eeshan Kalita
Asstt. Professor
of Molecular Biology & Biotechnology
Tezpur Central University
DBT Project ....management Practices.



(HEAD OF THE INSTITUTE)
(Signed and stamped)
Registrar
Tezpur University

Appendix A1

Sl. No.	Name of Sanctioned Equipment	Total value (₹)
1	Fluorescence microscope	1038836.00
2	Refrigerated programmable shaker	599420.00
3	BOD incubator & Biosafety hood	350000.00
	Total	**1988256.00**



Annexure A

Details of Manpower engaged (Financial Year 2015-16)

S. No.	Name & Designation of the Manpower engaged	Pay Scale provided	Date of Appointment	Salary Due	Salary disbursed	Difference, if any	Date of leaving, if any
1.	Mr. Bikash Kar Nath	₹ 25000/- + 10 % HRA	01.05.2015	₹ 3,02500/-	₹ 3,02500/-	NIL	NA

24.7.2017

(Project Investigator)

(Signed and stamped)

P.I. DBT Project
Towards..................management
Practices.
Dr. Eeshan Kalita
Asstt. Professor
Deptt. of Molecular Biology & Biotechnology
Tezpur Central University

(Head of the Institute)
(Signed and stamped)

Registrar
Tezpur University

22/8/17

(Finance Officer)

(Signed and stamped)

Finance Officer
Tezpur University

Annexure A

Details of Manpower engaged (Financial Year 2016-17)

S. No.	Name & Designation of the Manpower engaged	Pay Scale provided	Date of Appointment	Salary Due	Salary disbursed	Difference, if any	Date of leaving, if any
1.	Mr. Bikash Kar Nath	₹ 25000/- + 10 % HRA	01.05.2015	*₹ 3,30000/-	₹ 2,7500/-	₹ 3,02500/-	NA

*Due to unavailability of funds under the manpower head, the monthly emolument for J.R.F. @ ₹ 27,500 per month was not disbursed for the duration of **May 2016 to March 2017.** Thus an unpaid sum amounting to **₹ 3,02,500.00** stands as committed expenditure for the aforementioned financial term.

27.7.2017

(Project Investigator)

(Signed and stamped)

P.I. DBT Project
Towards.................management
Practices Dr. Eeshan Kalita
Asstt. Professor
Deptt. of Molecular Biology & Biotechnology
Tezpur Central University

22/8/17

(Head of the Institute)

(Signed and stamped)

Registrar
Tezpur University

22/8/17

(Finance Officer)

(Signed and stamped)

Finance Officer
Tezpur University

Annexure A

Manpower Staffing Details (Financial Year 2017-18)

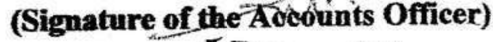
NAME OF THE PERSON	NAME OF THE POST	DATE OF JOINING	DATE OF LEAVING	TOTAL MONTHLY SALARY	TOTAL SALARY PAID DURING THE FINANCIAL YEAR	TOTAL SALARY PAID DURING THE PROJECT PERIOD
Miss Bikash Kar Nath	JRF with NET LS	01.05.2015	30.04.2017	₹ 27500.00	₹ 330000.00	₹ 660000.00

12. 11-2020

(Signature of the Principal Investigator)

r. Eeshan Kalita
Asstt. Professor
lecular Biology & Biotechnology
zpur Central University

P.I., DBT Project
Towards..................management
Practices.



12.11.2020

(Signature of the Accounts Officer)

Finance Officer
Tezpur University

(SIGNATURE OF THE HEAD OF THE INSTITUTE)

Registrar
Tezpur University

Appendix-B

Utilisation Certificate

(For the financial year ending 31st March 2016.)

(Rs. in Lakhs)

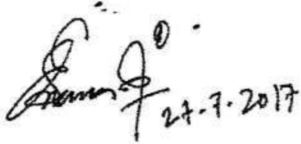
1.	Title of the Project/Scheme:	Towards identification isolation and characterization of Exobasidium vexans strains and their pathogenic determinants/effectors from Blister blight infested tea plantations of Assam and development of future road-map for effective management practices
2.	Name of the Organisation:	Tezpur University
3.	Principal Investigator:	Dr. Eeshan Kalita, Assistant Professor, Dept. of MBBT, TU
4.	Deptt. of Biotechnology sanction order No. & date of sanctioning the project:	BT/427/NE/TBP/2013 dated 25-03-2015
5.	Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given:	₹ 0
6.	Amount received from DBT during the financial year (*please give No. and dates of sanction orders showing the amounts paid)*:	₹ 3046000.00
7.	Other receipts/interest earned, if any, on the DBT grants:	₹ 0
8.	Total amount that was available for expenditure during the financial year (Sl. Nos. 5,6 and 7):	₹ 3046000.00
9.	Actual expenditure (excluding commitments) incurred during the financial year (statement of expenditure is enclosed):	₹ 363817.00
10.	Unspent balance refunded, if any *(Please give details of cheque No. etc.)*:	N/A
11.	Balance amount available at the end of the financial year:	₹ 2682183.00
12.	Amount allowed to be carried forward to the next financial year vide letter No. & date:	

1. Certified that the amount of **₹ 363817.00 (Rupees Three lakhs sixty three thousand eig hundred and seventeen only)** mentioned against col. 9 has been utilised on the project/sche for the purpose for which it was sanctioned and that the balance of **₹ 2682183.00 (Twenty s lakh eighty two thousand one hundred and eighty three only)** remaining unutilized at t end of the year has been surrendered to Govt. (vide No. ____________ dated ____________)/w be adjusted towards the grants-in-aid payable during the next year.

2. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanction have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see th the money was actually utilised for the purpose for which it was sanctioned.

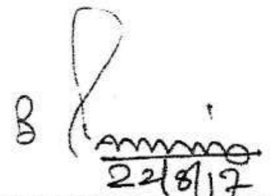
Kinds of checks exercised:

1. Cash Book
2. Ledgers
3. Vouchers
4. Bank Statement
5. Any Other



(PROJECT INVESTIGATOR)
(Signed and stamped)

P.I. DBT Project
Towards..................management
Practices.
Dr. Eeshan Kalita
Asstt. Professor
Deptt. of Molecular Biology & Biotechnology
Tezpur Central University



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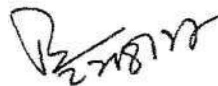
Tezpur University



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Tezpur University

Appendix-C

Statement of Expenditure referred to in para 9 of the Utilisation Certificate

Showing grants received the Department of Biotechnology and the expenditure incurred during the period from **1st April 2015 to 31st March 2016.**

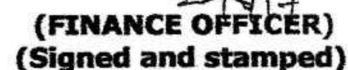
Sl. No.	Item	Unspent balance carried forward from Previous financial year 2014-15	Grants received from DBT during the financial year 2015-16	Other receipts/ interest earned if any, on the DBT grants	Total of the columns (2+3+4)	Expenditure (excluding commitments) incurred during the financial year 2015-16	Balance for the financial year 2015-16 (5-6)	Remarks (5-6)
	1	2	3	4	5	6	7	
1.	Non-Recurring							
i.	Equipment	0.00	1966000		1966000	0.00	1966000	
2.	Recurring							
i.	Human Resource*	0.00	330000	0	330000	302500	27500	
ii.	Consumables	0.00	600000		600000	15091	584909	
iii.	Travel	0.00	50000		50000	46226	3774	
iv.	Contingency	0.00	50000		50000	0.00	50000	
v.	Overheads	0.00	50000		50000	0.00	50000	
	TOTAL	0.00	3046000	0	3046000	363817	2682183	

*N.B. The first year release received vide order no. BT/427/NE/TBP/2013 dated 25-03-2015 was Rs. 30.46 lakhs.

27.7.2017

(PROJECT INVESTIGATOR)
(Signed and stamped)

P.I. DBT Project
Towards..................management
Practices.
Dr. Eeshan Kalita
Asstt. Professor
Deptt. of Molecular Biology & Biotechnology
Tezpur Central University



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Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)
(Signed and stamped)

Registrar
Tezpur University

3

Appendix-B

Utilisation Certificate

(For the financial year ending 31st March 2017.)

(Rs. in Lakhs)

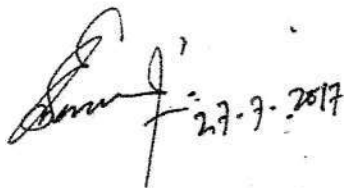
1. Title of the Project/Scheme: Towards identification isolation and characterization of Exobasidium vexans strains and their pathogenic determinants/effectors from Blister blight infested tea plantations of Assam and development of future road-map for effective management practices
2. Name of the Organisation: Tezpur University
3. Principal Investigator: Dr. Eeshan Kalita, Assistant Professor, Dept. of MBBT, TU
4. Deptt. of Biotechnology sanction order No. & date of sanctioning the project: BT/427/NE/TBP/2013 dated 25-03-2015
5. Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given: ₹ 0
6. Amount received from DBT during the financial year (*please give No. and dates of sanction orders showing the amounts paid*): ₹ 0
7. Other receipts/interest earned, if any, on the DBT grants: ₹ 0
8. Total amount that was available for expenditure during the financial year (Sl. Nos. 5,6 and 7): ₹ 2682183.00
9. Actual expenditure (excluding commitments) incurred during the financial year (statement of expenditure is enclosed): ₹ 753144.00
10. Unspent balance refunded, if any (*Please give details of cheque No. etc.*): N/A
11. Balance amount available at the end of the financial year: ₹ 1929039.00
12. Amount allowed to be carried forward to the next financial year vide letter No. & date:

1. Certified that the amount of **₹ 753144.00 (Rupees Seven lakhs fifty three thousand one hundred and forty four only)** mentioned against col. 9 has been utilised on the project/scheme for the purpose for which it was sanctioned and that the balance of **₹ 1929039.00 (Nineteen lakh twenty nine thousand thirty nine only)** remaining unutilized at the end of the year has been surrendered to Govt. (vide No. ____________ dated ____________)/will be adjusted towards the grants-in-aid payable during the next year.

2. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned.

Kinds of checks exercised:

1. Cash Book
2. Ledgers
3. Vouchers
4. Bank Statement
5. Any Other

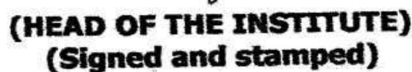


(PROJECT INVESTIGATOR)
(Signed and stamped)

Dr. Eeshan Kalita
Asstt. Professor
Deptt. of Molecular Biology & Biotechnology
Tezpur Central University
P.I. DBT Project
Towards..................Management Practices.

(FINANCE OFFICER)
(Signed and stamped)

Finance Officer
Tezpur University



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(Signed and stamped)

Registrar
Tezpur University

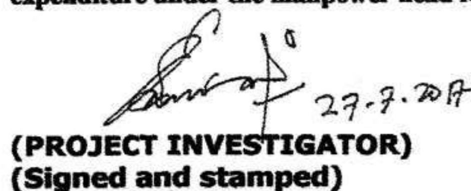
Appendix-C

Statement of Expenditure referred to in para 9 of the Utilisation Certificate

Showing grants received the Department of Biotechnology and the expenditure incurred during the period from **1st April 2016 to 31st March 2017.**

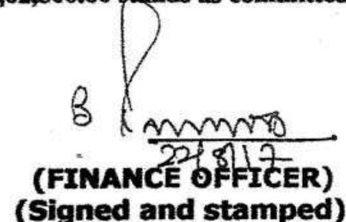
Sl. No.	Item	Unspent balance carried forward from Previous financial year 2015-16	Grants received from DBT during the financial year 2016-17	Other receipts/ interest earned if any, on the DBT grants	Total of the columns (2+3+4)	Expenditure (excluding commitments) incurred during the financial year 2016-17	Balance for the financial year 2016-17 (5-6)	Remarks (5-6)
	1	2	3	4	5	6	7	
1.	**Non-Recurring**							
i.	**Equipment**	1966000	0.00		1966000	0.00	1966000	
2.	**Recurring**							
i.	**Human Resource***	27500	0.00	0	27500	27500	0.00	
ii.	**Consumables**	584909	0.00		584909	598447	-13538	
iii.	**Travel**	3774	0.00		3774	34136	-30362	
iv.	**Contingency**	50000	0.00		50000	43085	6915	
v.	**Overheads**	50000	0.00		50000	49976	24	
	TOTAL	**2682183**	**0.00**	**0**	**2682183**	**753144**	**1929039**	

***N.B. The monthly emolument for J.R.F. @ ₹ 27,500 per month was not disbursed for the period May 2016- March 2017 due to unavailability of funds under the manpower head. The unpaid sum amounting to ₹ 3,02,500.00 stands as committed expenditure under the manpower head for the aforementioned financial term.**



(PROJECT INVESTIGATOR)
(Signed and stamped)

P.I. DBT Project
Towards..................management Practices.
Dr. Eeshan Kalita
Asstt. Professor
Deptt. of Molecular Biology & Biotechnology
Tezpur Central University



(FINANCE OFFICER)
(Signed and stamped)

Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)
(Signed and stamped)

Registrar
Tezpur University

Appendix-B1

Utilisation Certificate

(For the financial year ending 31st March 2018.)

(Rs. in Lakhs)

1. Title of the Project/Scheme: Towards identification isolation and characterization of *Exobasidium vexans* strains and their pathogenic determinants/effectors from Blister blight infested tea plantations of Assam and development of future road-map for effective management practices

2. Name of the Organisation: Tezpur University

3. Principal Investigator: Dr. Eeshan Kalita, Assistant Professor, Dept. of MBBT, TU

4. Deptt. of Biotechnology sanction order No. & date of sanctioning the project: BT/427/NE/TBP/2013 dated 02-11-2017

5. Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given: ₹ 2071671.00

6. Amount received from DBT during the financial year (*please give No. and dates of sanction orders showing the amounts paid)*: ₹ 1004000.00

7. Other receipts/interest earned, if any, on the DBT grants: ₹ 45004.00

8. Total amount that was available for expenditure during the financial year (Sl. Nos. 5,6 and 7): ₹ 3120675.00

9. Actual expenditure (excluding commitments) incurred during the financial year (statement of expenditure is enclosed): ₹ 536714.00

10. Unspent balance refunded, if any *(Please give details of cheque No. etc.)*: N/A

11. Balance amount available at the end of the financial year: ₹ 2583961.00

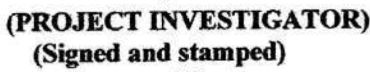
12. Amount allowed to be carried forward to the next financial year vide letter No. & date:

13. Certified that the amount of **₹ 536714.00 (Rupees Five lakhs thirty six thousand seven hundred and forteen only)** mentioned against col. 9 has been utilised on the project/~~scheme~~ for the purpose for which it was sanctioned and that the balance of **₹ 2583961.00 (Rupees Twenty five lakhs eighty three thousand nine hundred and sixty one only** remaining unutilized at the end of the year has been surrendered to Govt. (vide No. ____________ dated ____________)/will be adjusted towards the grants-in-aid payable during the next year.

14. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned.

Kinds of checks exercised:

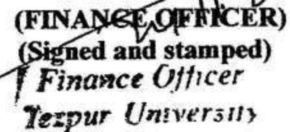
1. Cash Book
2. Ledgers
3. Vouchers
4. Bank Statement
5. Any Other



12·11·2020

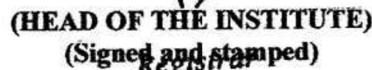
(PROJECT INVESTIGATOR)
(Signed and stamped)

Dr. Eeshan Kalita
Asstt. Professor
Deptt. of Molecular Biology & Biotechnology
Tezpur Central University
PI, DBT Project ....management Practices.



(FINANCE OFFICER)
(Signed and stamped)

Finance Officer
Tezpur University



(HEAD OF THE INSTITUTE)
(Signed and stamped)

Registrar
Tezpur University

(To be countersigned by the DBT Officer-in-charge)

Appendix-C1

Statement of Expenditure referred to in para 9 of the Utilisation Certificate

Showing grants received the Department of Biotechnology and the expenditure incurred during the period from **1st April 2017 to 31st March 2018**.

Sl. No.	Item	Unspent balance carried forward from Previous financial year 2016-17	Grants received from DBT during the financial year 2017-18	Other receipts/ interest earned if any, on the DBT grants	Total of the columns (2+3+4)	Expenditure (excluding commitments) incurred during the financial year 2017-18	Balance for the financial year 2017-18 (5-6)	Remarks (5-6)
	1	2	3	4	5	6	7	
1.	Non-Recurring							
i.	Equipment	1966000	0.00		1966000	0.00	1966000	
2.	Recurring			66632# + 45004##				
i.	Human Resource*	0.00	330000		330000	330000	0	
ii.	Consumables	-13538	600000		586462	135666	450796	
iii.	Travel	-30362	50000		19638	2852	16786	
iv.	Contingency	6915	24000	26000#	56915	36946	19969	
v.	Overheads	24	0.00	50000#	50024	31250	18774	
	TOTAL	1929039	1004000*	187636	3120675	536714	2583961	

* The second-year release received vide order no. BT/427/NE/TBP/2013 dated 02-11-2017 was Rs. 10.04 lakhs.

\# An amount of ₹76000 from total interest earned ₹142632 from the term 2015-2017 is adjusted as ₹50000 to overhead & ₹26000 to contingency vide letter no. BT/427/NE/TBP/2013 dated 02-11-2017 and the remaining amount ₹66632 is carried forward to the financial year 2017-18

\##Interest earned for the financial year 2017-18 is ₹45004

12-11-2020

(PROJECT INVESTIGATOR)
(Signed and stamped)
Dr. Eeshan Kalita
Asstt. Professor
Deptt. of Molecular Biology & Biotechnology
Tezpur Central University

(FINANCE OFFICER)
(Signed and stamped)
Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)
(Signed and stamped)
Registrar
Tezpur University

Appendix-B2

Utilisation Certificate

(For the financial year ending 31st March 2019)

(Rs. in Lakhs)

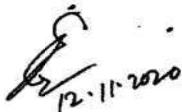
1. Title of the Project/Scheme: Towards identification isolation and characterization of Exobasidium vexans strains and their pathogenic determinants/effectors from Blister blight infested tea plantations of Assam and development of future road-map for effective management practices
2. Name of the Organisation: Tezpur University
3. Principal Investigator: Dr. Eeshan Kalita, Assistant Professor, Dept. of MBBT, TU
4. Deptt. of Biotechnology sanction order No. & date of sanctioning the project: BT/427/NE/TBP/2013 dated 02-11-2017
5. Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given: ₹ 2583961
6. Amount received from DBT during the financial year (*please give No. and dates of sanction orders showing the amounts paid*): ₹ 1602
7. Other receipts/interest earned, if any, on the DBT grants: ₹ 0
8. Total amount that was available for expenditure during the financial year (Sl. Nos. 5,6 and 7): ₹ 2585563.00
9. Actual expenditure (excluding commitments) incurred during the financial year (statement of expenditure is enclosed): ₹ 1446714.00
10. Unspent balance refunded, if any (*Please give details of cheque No. etc.*): N/A
11. Balance amount available at the end of the financial year: ₹ 1138849.00
12. Amount allowed to be carried forward to the next financial year vide letter No. & date:

13. Certified that the amount of **₹1446714.00 (Rupees Fourteen lakhs forty six thousand seven hundred and forteen only)** mentioned against col. 9 has been utilised on the project/~~scheme~~ for the purpose for which it was sanctioned and that the balance of ₹ **1138849.00 (Eleven lakh thirty eight thousand eight hundred and forty nine only)** remaining unutilized at the end of the year has been surrendered to Govt. (vide No. ____________ dated ____________)/will be adjusted towards the grants-in-aid payable during the next year.

14. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned.

Kinds of checks exercised:

1. Cash Book
2. Ledgers
3. Vouchers
4. Bank Statement
5. Any Other



(PROJECT INVESTIGATOR)
(Signed and stamped)

Dr. Eeshan Kalita
Asstt. Professor
Deptt. of Molecular Biology & Biotechnology
Tezpur Central University
P.I. DBT Project towards.................management Practices.

(FINANCE OFFICER)
(Signed and stamped)

Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)
(Signed and stamped)

Registrar
Tezpur University

(To be countersigned by the DBT Officer-in-charge)

Appendix-C2

Statement of Expenditure referred to in para 9 of the Utilisation Certificate

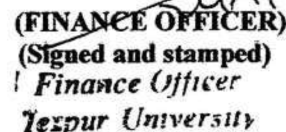
Showing grants received the Department of Biotechnology and the expenditure incurred during the period from **1st April 2018 to 31st March 2019.**

Sl. No.	Item	Unspent balance carried forward from Previous financial year 2017-18	Grants received from DBT during the financial year 2018-19	Other receipts/ interest earned if any, on the DBT grants	Total of the columns (2+3+4)	Expenditure (excluding commitments) incurred during the financial year 2018-19	Balance for the financial year 2018-19 (5-6)	Remarks (5-6)
	1	2	3	4	5	6	7	
1.	**Non-Recurring**							
i.	**Equipment**	1966000	0		1966000	949420	1016580	
2.	**Recurring**							
i.	**Human Resource***	0	0	111636* +	0	0	0	
ii.	**Consumables**	450796	0	1602#	450796	450712	84	
iii.	**Travel**	16786	0		16786	7840	8946	
iv.	**Contingency**	19969	0		19969	19970	-1	
v.	**Overheads**	18774	0		18774	18772	2	
	TOTAL	**2472325**	**0**	**113238***	**2585563**	**1446714**	**1138849**	

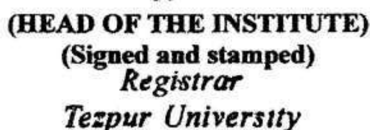
*Carried forward from interest earned up to financial year 2018 (2015-2018)
#Interest earned for the financial year 2018-19 is ₹1602

12.11.2020

(PROJECT INVESTIGATOR)
(Signed and stamped)
Dr. ...
Asstt. Professor P.I. DBT Project
Dept. of Molecular Biology & Biotechnology ... management
Tezpur Central University ... practices.


12.11.20

(FINANCE OFFICER)
(Signed and stamped)
Finance Officer
Tezpur University



(HEAD OF THE INSTITUTE)
(Signed and stamped)
Registrar
Tezpur University

Appendix-B3

Utilisation Certificate

(For the financial year ending 31st March, 2020)

(Rs. in Lakhs)

1. Title of the Project/Scheme: Towards identification isolation and characterization of Exobasidium vexans strains and their pathogenic determinants/effectors from Blister blight infested tea plantations of Assam and development of future road-map for effective management practices
2. Name of the Organisation: Tezpur University
3. Principal Investigator: Dr. Eeshan Kalita, Assistant Professor, Dept. of MBBT, TU
4. Deptt. of Biotechnology sanction order No. & date of sanctioning the project: BT/427/NE/TBP/2013 dated 25-03-2015
5. Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given: ₹ 1138849
6. Amount received from DBT during the financial year (*please give No. and dates of sanction orders showing the amounts paid)*: ₹ 0
7. Other receipts/interest earned, if any, on the DBT grants: ₹ 1602
8. Total amount that was available for expenditure during the financial year (Sl. Nos. 5,6 and 7): ₹ 1140451.00
9. Actual expenditure (excluding commitments) incurred during the financial year (statement of expenditure is enclosed): ₹ 0.00
10. Unspent balance refunded, if any *(Please give details of cheque No. etc.)*: N/A
11. Balance amount available at the end of the financial year: ₹ 1140451.00
12. Amount allowed to be carried forward to the

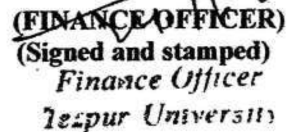
next financial year vide letter No. & date:

13. Certified that the amount of **₹ 0.00 (Rupees Zero only)** mentioned against col. 9 has been utilised on the project/~~scheme~~ for the purpose for which it was sanctioned and that the balance of **₹ 1140451.00 (Eleven lakh forty thousand four hundred and fifty one only)** remaining unutilized at the end of the year has been surrendered to Govt. (vide No. ___________ dated ___________)/will be adjusted towards the grants-in-aid payable during the next year.

14. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned.

Kinds of checks exercised:

1. Cash Book
2. Ledgers
3. Vouchers
4. Bank Statement
5. Any Other



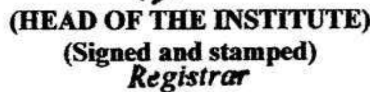
(PROJECT INVESTIGATOR)
(Signed and stamped)

Dr. Leshan Kalita
Asstt. Professor
Deptt. of Molecular Biology & Biotechnology
Tezpur Central University

DBT Project ....management Practices.

(FINANCE OFFICER)
(Signed and stamped)

Finance Officer
Tezpur University



(HEAD OF THE INSTITUTE)
(Signed and stamped)

Registrar
Tezpur University

(To be countersigned by the DBT Officer-in-charge)

Appendix-C3

Statement of Expenditure referred to in para 9 of the Utilisation Certificate

Showing grants received the Department of Biotechnology and the expenditure incurred during the period from **1st April 2019 to 31st March 2020.**

Sl. No.	Item	Unspent balance carried forward from Previous financial year 2018-19	Grants received from DBT during the financial year 2019-20	Other receipts/ interest earned if any, on the DBT grants	Total of the columns (2+3+4)	Expenditure (excluding commitments) incurred during the financial year 2019-20	Balance for the financial year 2019-20	Remarks (5-6)
	1	2	3	4	5	6	7	
1.	Non-Recurring							
i.	Equipment	1016580	0.00		1016580	0.00	1016580	
2.	Recurring							
i.	Human Resource*	0	0.00	113238* + 1602#	0.00	0.00	0.00	
ii.	Consumables	84	0.00		84	0.00	84	
iii.	Travel	8946	0.00		8946	0.00	8946	
iv.	Contingency	-1	0.00		-1	0.00	-1	
v.	Overheads	2	0.00		2	0.00	-2	
	TOTAL	1025611	0.00	114840	1140451	0.00	1140451	

*Carried forward from interest earned up to financial year 2019 (2015-2019)
#Interest earned for the financial year 2019-20 is ₹1602

12-11-2020
(PROJECT INVESTIGATOR)
(Signed and stamped)
Dr. Eeshan Kalita
Asstt. Professor
Deptt. of Molecular Biology & Biotechnology
Tezpur Central University

(HEAD OF THE INSTITUTE)
(Signed and stamped)
Registrar
Tezpur University

12-11-2020
(FINANCE OFFICER)
(Signed and stamped)
Finance Officer
Tezpur University

Appendix-B4

Utilisation Certificate

(For the financial the period 1st April 2020 to 30th June 2020.)

(Rs. in Lakhs)

1. Title of the Project/Scheme: Towards identification isolation and characterization of Exobasidium vexans strains and their pathogenic determinants/effectors from Blister blight infested tea plantations of Assam and development of future road-map for effective management practices
2. Name of the Organisation: Tezpur University
3. Principal Investigator: Dr. Eeshan Kalita, Assistant Professor, Dept. of MBBT, TU
4. Deptt. of Biotechnology sanction order No. & date of sanctioning the project: BT/427/NE/TBP/2013 dated 25-03-2015
5. Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given: ₹ 1140451
6. Amount received from DBT during the financial year (*please give No. and dates of sanction orders showing the amounts paid*): ₹ 0
7. Other receipts/interest earned, if any, on the DBT grants: ₹ 166
8. Total amount that was available for expenditure during the financial year (Sl. Nos. 5,6 and 7): ₹ 1140617.00
9. Actual expenditure (excluding commitments) incurred during the financial year (statement of expenditure is enclosed): ₹ 1070086.00
10. Unspent balance refunded, if any (*Please give details of cheque No. etc.*): N/A
11. Balance amount available at the end of the financial year: ₹ 70531.00
12. Amount allowed to be carried forward to the next financial year vide letter No. & date:

13. Certified that the amount of **₹ 1070086.00 (Rupees Ten Lakh seventy thousand and eighty six only)** mentioned against col. 9 has been utilised on the project/~~scheme~~ for the purpose for which it was sanctioned and that the balance of **₹ 70531.00 (Seventy thousand five hundred and thirty one)** remaining unutilized at the end of the year has been surrendered to Govt. (vide No. ____________ dated ____________)/will be adjusted towards the grants-in-aid payable during the next year.

14. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned.

Kinds of checks exercised:

1. Cash Book
2. Ledgers
3. Vouchers
4. Bank Statement
5. Any Other

12.11.2020

(PROJECT INVESTIGATOR)
(Signed and stamped)

Dr. Eeshan Kalita
Asstt. Professor
Deptt. of Molecular Biology & Biotechnology
Tezpur Central University

24.11.2020

(FINANCE OFFICER)
(Signed and stamped)

Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)
(Signed and stamped)

Registrar
Tezpur University

(To be countersigned by the DBT Officer-in-charge)

Appendix-C4

Statement of Expenditure referred to in para 9 of the Utilisation Certificate

Showing grants received the Department of Biotechnology and the expenditure incurred during the period from **1st April 2020 to 30th June 2020.**

Sl. No.	Item	Unspent balance carried forward from Previous financial year 2019-20	Grants received from DBT during the financial year 2020-21	Other receipts/ Interest earned if any, on the DBT grants	Total of the columns (2+3+4)	Expenditure (excluding commitments) incurred during the financial term ending 30th June 2020	Balance for the financial term ending 30th June 2020	Remarks (5-6)
	1	2	3	4	5	6	7	
1.	**Non-Recurring**							
i.	Equipment	1016580	0.00	114840* + 166#	1016580	1020084##	-3504**	
2.	**Recurring**							
i.	Human Resource*	0	0.00		0.00	0.00	0.00	
ii.	Consumables	84	0.00		84	0.00	84	
iii.	Travel	8946	0.00		8946	0.00	8946	
iv.	Contingency	-1	0.00		-1	0.00	-1	
v.	Overheads	2	0.00		2	50002##	-50000**	
	TOTAL	**1025611**	**0.00**	**115006****	**1140617**	**1070086**	**70531**	

*Carried forward from interest earned up to financial year 2020 (2015-2020)

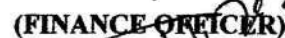
#Interest earned for the financial year 2020-21 upto 30th June 2020 is ₹166

##A total amount of ₹1038836 via wire transfer from equipment head paid as advance in the financial year 2017-2018 for equipment has been re-adjusted from equipment head (₹1020084) and Overhead (₹18752). An amount of ₹18752 had to be readjusted from overhead grant due to change in currency exchange rate.

**The pending dues in the Overhead (₹50000) and Equipment head (₹3504) amounting to ₹53504 has been adjusted from the remaining interest earned (2015-2021) of amount ₹115006

12.11.2020

(PROJECT INVESTIGATOR)
(Signed and stamped)
Dr. Eeshan Kalita
Asstt. Professor
Deptt. of Molecular Biology & Biotechnology
Tezpur Central University



(FINANCE OFFICER)
(Signed and stamped)
Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)
(Signed and stamped)
Registrar
Tezpur University

Consolidated statement for final settlement of account for the DBT project "TOWARDS IDENTIFICATION........MANAGEMENT PRACTICES" for the financial term dated 1st May 2015 to 30th June 2020

Item	Grants received from DBT						Expenditure as per latest Statement of expenditure (SOE)							
	1st May 2015 to 31st March 2016	1st May 2016 to 31st March 2017	Interest gained (2015 -2017)	1st May 2017 to 31st March 2018	Interest gained (2017-30th June 2020)	Grand total	1st May-2015 to 31st March 2016	1st April 2016 to 31st March 2017	1st April 2017 to 31st March 2018	1st April 2018 to 31st March 2019	1st April 2019 to 31st March 2020	1st April 2020 to 30th June 2020	Total	Balance as per released fund
Head	(₹)	(₹)	(₹)	(₹)	(₹)	(₹)	(₹)	(₹)	(₹)	(₹)	(₹)	(₹)	(₹)	(₹)
Equipment	1966000	0.00		0.00		1966000	0.00	0.00	0.00	949420	0.00	1020084	1969504	-3504
Manpower	330000	0.00	66632	330000		660000	302500	27500	330000	0	0.00	0.00	660000	0.00
Consumable	600000	0.00		600000	48374	1200000	15091	598447	135666	450712	0.00	0.00	1199916	84
Travel	50000	0.00		50000		100000	46228	34136	2852	7840	0.00	0.00	91054	8946
Contingency	50000	0.00	26000	24000		100000	0.00	43085	36946	19970	0.00	0.00	100001	-1
Overhead	50000	0.00	50000	0.00		100000	0.00	49976	31250	18772	0.00	50002	150000	-50000
Total	3046000	0.00	142632	1004000	48374	4241006	363817	753144	536714	1446714	0.00	1070086	4170475	

Total unspent Balance	70531.00

Remarks: A total amount of ₹1038836 via wire transfer from equipment head paid as advance in the financial year 2017-2018 for equipment has been re-adjusted from equipment head (₹1020084) and Overhead (₹18752) in financial year 2020-2021. An amount of ₹18752 had to be readjusted from overhead grant due to change in currency exchange rate. The pending dues in the Overhead (₹50000) and Equipment head (₹3504) amounting to ₹53504 has been adjusted from the interest earned from financial year 2015-2021 of amount ₹115006 (₹66632 + ₹48374).

12·11·2020

E. Kalita
Principal Investigator DBT Project
Tezpur University

Dr. Eeshan Kalita
Asstt. Professor
Deptt. of Molecular Biology & Biotechnology

P.I. DBT Project
Towards..................management Practices.

Finance Officer
Tezpur University
Finance Officer
Tezpur University

Registrar
Tezpur University
Registrar
Tezpur University