



## Etiology of sooty blotch disease of *Aegle marmelos* and its management

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**ABSTRACT:** *Aegle marmelos* (L) Correa. recognized as bael is widely grown in the Eastern and Northern states of India. Incidence of superficial smudgy fungal blotch symptoms were observed on bael fruits in our experimental farm located in the state of Odisha, Eastern India (20°14' N, 85°46' E). The fungal blotch colonies on the fruit skin reduced the visual appeal of the fruits and thereby drastically reduced the market value and saleability of fruits. Etiology of fungi growing on the waxy layer of fruits resulting sooty blotch symptoms were investigated. Among different mycelial types observed on fruits, ramose colony type was observed as predominant (up to 80 %) followed by fuliginous and punctate type of colonies. Representative colonies were subjected to isolation; cultures were purified and analysed based on morphology and sequences generated with nuclear ribosomal genetic marker, the Internal Transcribed Spacer (ITS) nr DNA region and proved for Koch's postulates. The three fungal isolates were identified based on ITS phylogeny viz., *Zasmidium* sp. (ISO141), *Passalora* sp. (ISO211) and *Pseudocercospora* sp. (ISO232) however assigned putative status as the cultures were sterile. Among the various pre-harvest management modules evaluated in an effort to produce blemish-free bael fruit, the field spray of 0.3 percent copper oxy chloride (first spray at the lemon stage, second and third spray at 15 and 30 days after the first spray, and the last spray at 30 days before harvest) resulted 95-97 percent blotch control in two varieties of bael CISH-B1 and NB-5 taken for study.

**Keywords:** Sooty blotch disease, Bael, *Aegle marmelos*, etiology, management, India

### INTRODUCTION

*Aegle marmelos*, commonly known as bael, has also been designated with several appellations like wood apple, Bengal quince, stone apple, holy fruit tree, etc. In India, it is abundantly distributed in Himalayan tracts, Eastern, Central and South India. Almost all parts of the bael tree have immense medicinal and nutritional value (Kumar and Nath 2010). A fully-grown (10-12 years old) grafted bael tree yields normally 150-200 fruits under a good crop care. Bael flowering starts during May, and after fruit set, bael fruit takes 10-11 months for ripening (<https://www.krishisewa.com/crop-production/372-bael.html>). CISH- B-1, CISH- B-2, Narendra Bael-5, Narendra bael-7, Narendra Bael-9, Pant Shivani, Pant Urvashi, Pant Aparna and Pant Pant Sujata are some of the popular bael varieties released for commercial fruits cultivation by research organizations in India.

Bael is reported to be affected by several diseases like bacterial spot by *Xanthomonas campestris* pv. *bilvae* (Patel *et al.* 1951), shell rot by *Syncephalastrum racemosum* (Mishra *et al.*, 2016), black mildew by *Schiffnerula girijae* (Gautam 2014) and leaf spot by *Alternaria alternata* (Maurya *et al.* 2016), etc. Fruits of almost all bael accessions and varietal collection in

our experimental farm were found to be covered with smudgy fungal growth wherein fruits became almost black at the of time maturity. The affected fruits lost their visual appeal and therefore, the saleability of fruits was greatly reduced. However, it is pertinent to note that there is no adverse impact on fruit pulp quality. These types of symptoms were reported to occur on pomaceous fruits by a group of epiphytic fungi 'sooty blotch flyspeck' (Sutton and Sutton 1994; Batzer *et al.*, 2005). Sooty blotch is more often confused with sooty moulds. Sooty blotch diseases are alike to sooty moulds but are not associated with phloem-feeding insects hence sooty blotch development does not depend upon honeydew excretion of sap sucking insects. Review of literature revealed that sooty blotch diseases are widespread throughout the world occurring on different fruits (Batzer *et al.* 2005 and Gleason *et al.*, 2011). Blemishes on apples due to sooty blotch led to a reduction in market value which in turn cause severe economic loss for growers (Johnson *et al.* 1997). Sooty blotch is also reported as an emerging problem on mango in India (IIHR annual report, 2016). Earlier the number of fungi causing sooty blotch was under-estimated but now studies clearly revealed the involvement of a diverse range of pathogens in the sooty blotch fungal complex. Now world-wide the sooty

**Table 1. Effect of fungicide spray for the management of blackening caused by sooty blotch disease in Bael var CISH-B1**

Treatment	2016-17		2017-18	
	Mean sooty blotch grade (0-5 scale)	Per cent reduction of fruit blackening over control	Mean sooty blotch grade (0-5 scale)	Per cent reduction of fruit blackening over control
Carbendazim (0.2percent) + Captan (0.1percent)	2.2±0.093	54.70	2.18±0.020	58.47
Thiophanate methyl (0.2percent) + Captan (0.1percent)	0.47±0.032	90.23	0.50±0.071	89.51
Copper oxychloride (0.3 percent)	0.17±0.010	96.35	0.12±0.020	97.48
Mancozeb (0.2percent)	3.38±0.171	29.03	3.26±0.103	31.63
Control	4.78±0.080	0.00	5.00±0.000	-
CD (5%)	0.27		0.17	
CV	9.56		5.88	

Note: Values are reported as means ± SE

Replication: 5 trees per treatment; Data were taken randomly on 20 fruits/tree

blotch flyspeck (SBFS) complex has extended to more than hundred named and putative fungal species as per a very recent review (Gleason *et al.* 2019). So far, no studies have yet been carried out on sooty blotch disease in bael and strategies to manage them. Hence, the present investigation was carried out with an aim to determine the causal fungi involved in causing sooty blotch disease of bael in the prevailing climate of eastern coastal regions of India, as well as to develop a strategic management module to reduce the blackening of bael fruits caused by sooty blotch fungal complex.

## MATERIALS AND METHODS

This investigation was carried out during 2015-2019 at the experimental farm of IIHR-Central Horticultural Experiment Station (20°14' 17 N, 85°46' E), located in the state of Odisha. This region is characterised by a hot and humid tropical climate with annual rainfall of 1550 mm with relatively long spell of rainfall (June–September).

### Symptomatology and mycelial types on fruits

As sooty blotch fungi known to exhibit different mycelial types on fruit surface, the prevalent mycelial type on bael fruit was identified with handheld and or stereo zoom microscope. Mycelial type is a colony morphology on fruit and it is a constant character within every SBFS species. At present, the recognized mycelial types of SBFS include punctate, ramose, fuliginous, speck, discrete, compact speck, ridged honeycomb,

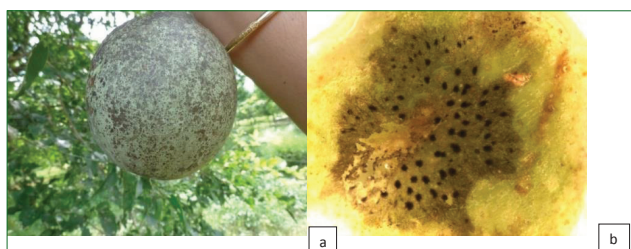
flyspeck and fleck (Gleason *et al.* 2011). Data on mycelial types were recorded from 30 fruits collected arbitrarily from trees of bael var. NB-5 (n=10).

### Isolation

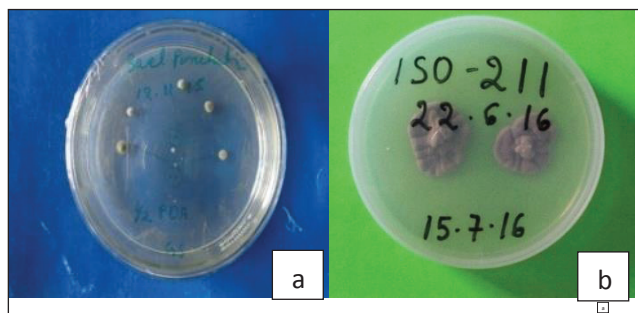
Fruits of bael cv. NB-5 displaying signs of sooty blotch were collected and washed in tap water gently for 4-5 mins. Surface sterilization of fruit was not useful since these epiphytic fungi were readily killed by surface sterilizing agents. Hence, fruits were gently cleaned with sterile water using sterile cotton swabs under aseptic conditions and fruits were left to dry on sterile filter paper on laminar hood. Then the blotch colonies were marked and every single knot-like fungal structures or mycelial growth (for fuliginous type) was chosen and picked with a sterile scalpel and transferred to half-strength PDA as well as acidified water agar by (adding lactic acid @ 7 drops for 500 ml media) and incubated at 28±2°C for 2 weeks and observed periodically for fungal growth. As the growth of SBFS fungi was very slow, the fast-growing fungal colonies which were saprophytic were not chosen for subculturing. The emerging hypha from acidified water agar as well as half-strength PDA were sub cultured on PDA plates and observed for uniformity. Then the representative fungal isolates exhibiting similar colony morphology were chosen and taken for further studies.

### Pathogenicity

Selected seven representative fungal isolates were



**Fig. 1.** Sooty blotch on bael fruits (a); Ramose mycelial types of sooty blotch fungi on bael fruits (b) (Bar=1000µm).



**Fig 2.** Isolation of blotch fungi from bael on water agar (a); Pure culture *Passalora* sp. ISO 211(b)

subjected to Koch's postulates. Modified Koch's postulates described for epiphytic pathogens by Batzer *et al.*, (2015) was adapted in this study. Blemish-free healthy lemon size bael fruits were selected and were surface-disinfested in the orchard using an alcohol spray and washed with sterile distilled water thrice. The fungal propagules prepared from respective pure cultures were swabbed onto the fruit surface with sterile cotton balls, and the inoculated fruits were covered immediately with double-layer polypropylene sleeves followed by polythene covers. Fruits were sprayed with sterile distilled water daily with a small mist sprayer to ensure wetness on the fruit surface. The fruits that received no inoculation, served as control. The fruits were observed for signs of sooty blotch at periodical interval. The fruits were inoculated during June month and after three months when the signs of sooty blotch were visible to the naked eye, the fruits were brought to the laboratory, bags were separated, mycelial type appeared was compared with that of the original isolate.

### Morphology of sooty blotch fungi *in vitro*

The three fungal isolates which produced sooty blotch symptoms were characterized for morphological parameters. The three isolates were grown on PDA by placing 8 mm diameter mycelial plug of respective cultures from month-old colonies and incubated at  $28 \pm 2^\circ\text{C}$  in an alternate cycle of dark and light for 30 days as they were very slow growing in nature. After one month of growth on PDA, the colony texture,

colour, growth each fungal colony was documented and observed under microscope (BX 53 Olympus make) for micromorphological characters.

### Molecular identification by ITS-rDNA sequence analysis and phylogeny

The three fungal isolates such as ISO141, ISO211 and ISO 232 were subject to ITS-rDNA sequence analysis to determine their identity. Mycelial disc from respective pure cultures of the said isolates were aseptically transferred to the potato-dextrose broth and incubated for 20 days at  $28 \pm 2^\circ\text{C}$  without any disturbance. The mycelial mat was harvested and DNA was extracted by the protocol of Doyle and Doyle 1990. The PCR was carried out using standard universal fungal primer pairs ITS1/ITS4 (White *et al.* 1990). Each PCR reaction consists of total reaction volume of  $25\mu\text{l}$  and the reaction was performed in an Eppendorf Thermal Cycler (made in Germany) with reaction cycles of initial denaturing for 2 min at  $94^\circ\text{C}$  followed by 35 cycles of 1 min at  $94^\circ\text{C}$ , 1 min at  $55^\circ\text{C}$ , 2 min at  $72^\circ\text{C}$ , and a final extension of 10 min at  $72^\circ\text{C}$ . Amplified products were separated on agarose gel (1.5 % w/v) alongside a 1.0 kb marker (Thermo Scientific, USA) for about 1-1.5 hours. After confirmation, the amplified PCR products were subsequently sent to sequencing (Eurofins Scientific India Pvt Ltd, Bengaluru). Resulted sequences were edited and aligned using Clustal-W (Kumar *et al.* 2016). Then, BLASTn search was performed in the NCBI GenBank database against available nucleotide sequences (<https://blast.ncbi.nlm.nih.gov>). The preliminary identity of each fungal isolate was decided based on the nearest match of the acquired sequence to the query sequence in the GenBank database (Lim *et al.* 2019) and isolates were given with putative status., as the cultures were observed to be sterile. Putative species is a provisional taxonomic unit, chosen based on several lines of available evidence which has not yet allotted a Latin binomial (Gleason *et al.* 2011). A phylogenetic tree based on the ITS sequences was made using Mega 7 software (Kumar *et al.* 2016).

### Scanning electron microscopy

The blotch infected bael fruit peels were subjected to scanning electron microscopy (SEM) studies to assess the damage to the fruit surface and examined under a scanning electron microscope TM 3030 Plus tabletop microscope (Hitachi high Tech corporation).

### Management of sooty blotch disease of bael under field condition

Ten years old bael plants of cv CISH-B1 and NB-5 maintained under uniform cultural practices were selected for the present study. Based on the review of literature,

**Table 2. Effect of fungicide spray for the management of blackening caused by sooty blotch disease in bael var. NB-5**

Treatment	2016-17		2017-18	
	Mean sooty blotch disease grade (0-5 scale)	Percent reduction of fruit blackening over control	Mean sooty blotch disease grade (0-5 scale)	Percent reduction of fruit blackening over control
Carbendazim (0.2percent) + Captan (0.1percent)	2.05±0.022	57.01	2.14±0.051	55.12
Thiophanate methyl (0.2percent) + Captan (0.1percent)	0.40±0.017	91.65	0.46±0.01	90.35
Copper oxychloride (0.3 percent)	0.11±0.013	97.94	0.19±0.079	96.02
Mancozeb (0.2percent)	3.14±0.093	34.06	3.28±0.183	29.95
Control	4.77±0.077	0.00	5.0±0.000	0.00
CD (5%)	0.16		0.29	
CV	5.60		9.70	

Note: Values are reported as means  $\pm$  SE

Replication: 5 trees per treatment; \*Data were taken on 20 fruits collected randomly per tree

fungicides shown effectiveness against sooty blotch disease on other crops such as copper oxychloride 50WP, captan 50WP, thiophanate methyl 72WP, mancozeb 75WP, carbendazim 50WP were taken for study. The treatments were T-1: carbendazim (0.2percent) + captan (0.1percent); T-2: thiophanate methyl (0.2percent) + captan (0.1percent); T-3: copper oxychloride (0.3 percent); T-4: mancozeb (0.2) and T-5: control. At the time of fungicide spraying, fruits were sprayed till runoff to ensure complete coverage with fungicide solution. The first fungicidal spray was done as a preventive spray when fruits were in the lemon stage before the onset of the southwest monsoon. The second and third spray was given 15 and 30 days after the first spray, and the last spray at 30 days before harvest. Each treatment was replicated three times @3 trees per replication. Plants subjected to treatment were observed regularly. At harvest, data were taken randomly on 20 fruits per tree including control and brought to the laboratory for disease assessment. Disease assessment was done visually for individual fruit with a 0-5 scale as described in the above section. The experimental data were subjected to the Kruskal Wallis one-way analysis of variance Test and analysis of variance (ANOVA) using Minitab version 20.1.2.

## RESULTS

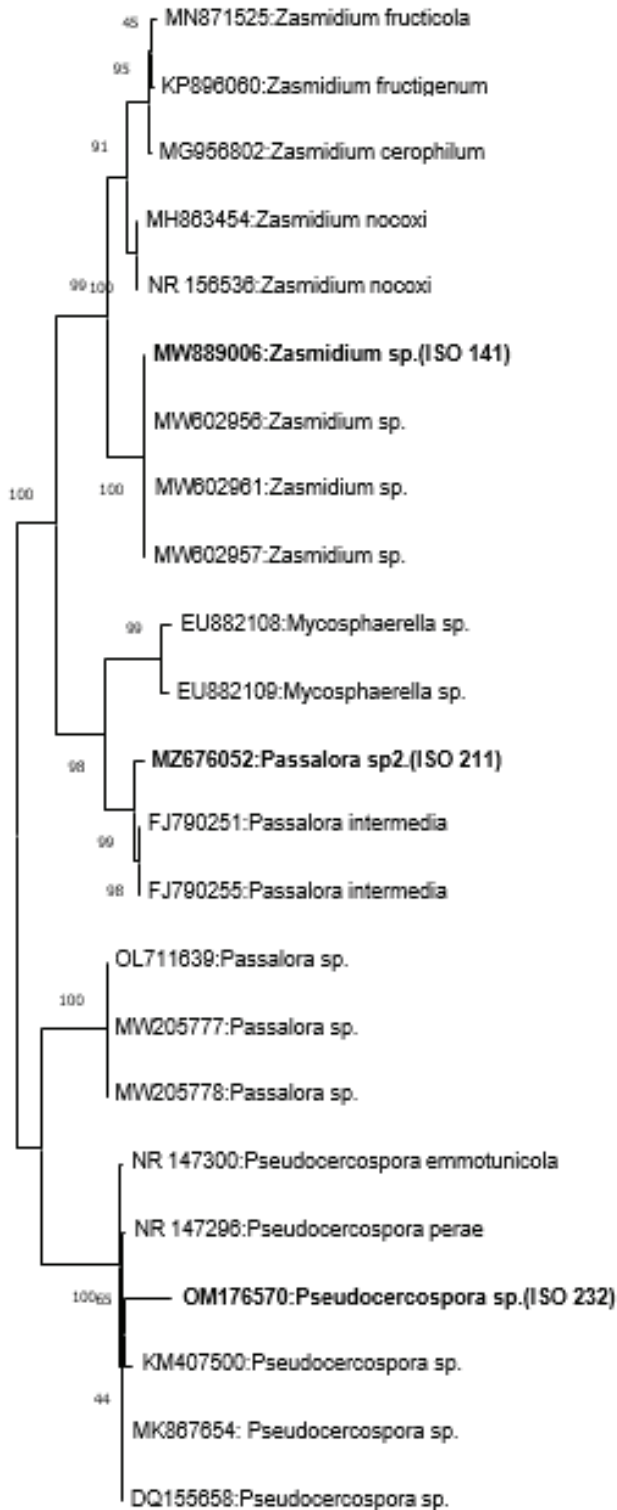
### Symptomatology and mycelial types

Sooty blotch fungi colonize the waxy layer of fruits, which was confirmed by studying the sign associated with the bael fruits. The fungi that cause them, grew on the surface of waxy layer of fruits and did not damage the

fruit itself. Initially, the blotch colonies were isolated in nature, but as the disease progressed, the colonies become enlarged with indefinite outlines which coalesced to cover large areas of the fruit and caused complete superficial fruit blackening. The blotches ultimately became duskier and denser and the whole affected fruit area turned black (Fig.1a). The variation in shapes and colour of blotch colonies was attributed to the differences among the sooty blotch fungal complex causing the blackening and environmental conditions. Even though the occurrence of flyspeck was reported to occur on apples and other fruits, flyspeck was not observed on bael fruits during our study.

Among various mycelial types commonly reported to be produced by sooty blotch fungal complex, on bael fruits, ramose colonies were observed as predominant (up to 80 %) followed by punctate and fuliginous type. The ramose mycelial types displayed branching with a strong radial growth pattern (Fig 1b). The fuliginous type produced smudgy colony type covering large area of fruits with or without defined margins and punctate mycelial type formed dark dots/specks interconnected by mycelium.

Isolation of blotch fungi on acidified water agar yielded fair results (Fig 2a) even though routine method of isolation on PDA was not helpful due to the slow growing nature of blotch fungi as they are easily overgrown by saprophytes. However, subsequent sub culturing was done on PDA, but sooty blotch fungi took almost 30 days to grow into an inch colony diameter. The colonies ISO141 on PDA were observed to be very leathery and



**Fig 4. Phylogenetic relationship of fungi associated with sooty blotch complex in Bael such as Isolate ISO 141, ISO211 and ISO 232 and reference isolates retrieved from GenBank, Branch support (bootstrap values) given on the branches.**

heaped, varied from dark grey, deep brown to black, slightly elevated at the centre, the surface appeared to be grey to dark grey, in reverse was iron black, wrinkled and crumpled. ISO211 (Fig 2b) and ISO 232 both produced grey leathery colonies. However, none of the three fungi sporulated on cultures and the culture was completely sterile in spite of efforts to induce sporulation in oatmeal agar, cornmeal agar or PDA amended with host extracts. It is in agreement with earlier observations made by Gleason *et al.* (2011) wherein many SBFS fungal species sporulate seldom or not at all on fruit surface as well as on in culture media, which frustrates description of associated fungal species morphologically. The three representative isolates ISO141, ISO211 and ISO 232 were subjected to ITS-rDNA analysis to assign putative status. These three fungal isolates produced similar sooty blotch signs on bael fruits upon artificial inoculation. The representative images of modified Koch’s postulates study were depicted in Fig 3a and 3b.

**Molecular identification by ITS-rDNA sequence analysis**

The partial ITS sequences of three fungi described in this study were deposited in NCBI GenBank and received accession numbers. Based on a mega blast search in GenBank nucleotide database, the nearest hits of ITS sequence of ISO 141-MW889006 shared 98.80 identity with *Zasmidium syzygyi* (GenBank AccessionNo. NR111826) hence given the putative status of *Zasmidium* sp. Likewise, ISO211 (NCBI Accession No.MZ676052 has shown 98.91 percent identity with *Passalora intermedia* GenBank Accession No.FJ790251 and FJ790255) hence assigned the putative status as *Passalora* sp. Similarly, ISO232 (Accession No.OM176570) has shown 99.75% percent similarity with *Pseudocercospora* sp. available in (GenBank Accession No. MH059763) hence assigned the putative status of *Pseudocercospora* sp. As all these three fungi causing the sooty blotch on bael were sterile, Latin binomial could not be assigned hence we have given only putative status. Phylogenetic relationship of fungi associated with sooty blotch complex such as *Zasmidium* sp, (ISO141), *Passalora* sp. (ISO211) and *Pseudocercospora* sp. (ISO 232) in bael and relevant isolates retrieved from GenBank, inferred by the neighbor-joining method using ITS sequences. Branch support (bootstrap values) were given on the branches (Fig.4).

**Scanning electron microscopy**

The scanning electron microscopy (SEM) images of infected bael fruit’s peel revealed that the blotch fungi grow on a waxy layer (Fig 5) but did not appear to



**Fig. 3. Pathogenicity evaluation on bael, Control fruits were bagged but not inoculated (a) ISO 211 inoculated on bael fruit, wherein fruits were bagged and inoculated (b). Isolation, identification and pathogenicity test**

dissolve the waxy layer. Further, it was also confirmed that during storage it did not result in any kind of skin shrinkage of bael fruit. Hence it is concluded that sooty blotch fungi resulted in superficial colonization of bael fruits.

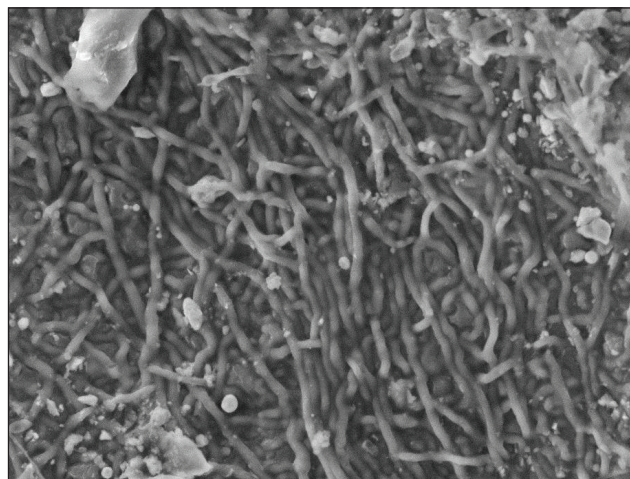
#### **Management of sooty blotch disease of bael under field condition**

Our investigation over a period of four year revealed that sooty blotch infection initiated during onset of southwest monsoon i.e., during last week of July and by the end of September, the bael fruits were almost black. Hence prophylactic fungicide spray was initiated when the fruit were in lemon size.

Kruskal Wallis Test revealed significant differences on four levels of treatment with a p-value of 0.0001 and the mean rank was different across all treatments. Among the four fungicides/fungicide combinations evaluated under field condition on bael for managing the blackening caused by sooty blotch fungal complex, four-time field sprays of 0.3 percent copper oxychloride (first spray at the lemon stage, second and third spray at 15 and 30 days after the first spray, and the last spray at 30 days before harvest) provided around 96-97 percent blotch control in both the varieties CISH-B1 and NB-5 which were evaluated for two years 2016-17 and 2017-18 (Table 1&2; Fig. 6&7). It was followed by spray with thiophanate methyl (0.2percent) + captan (0.1percent) which provided 89-91 percent blotch control over unsprayed control (Fig 7). However, carbendazim (0.2 percent) + captan (0.1percent) and mancozeb (0.1 percent) spray could not offer a satisfactory level of blotch control.

## **DISCUSSION**

In the current study, it has been established that the blackening in bael was caused by sooty blotch fungal complex and superficial fungal blackening was not found to be associated with phloem-feeding insects. Although these groups of fungi did not affect the development of fruit and did not result in direct yield loss, but severely impact the eye appeal and market value of fruits. In apple, sooty blotch was reported to reduce the market value to 90 percent and above (Williamson and Sutton 2000; Batzer *et al.* 2002). In a study conducted in apple orchards located in North Carolina, United States, Sutton and Sutton (1994) observed that the punctate mycelial type was most predominant in areas where sooty blotch severity was severe, however, the punctate mycelial type increased with cumulative hours of high humidity and the ramose mycelial type increased with increasing rainfall and temperature which is prevalent in the Coastal Plain region. Similarly, ramose mycelial type was observed predominantly on bael fruits in our study as our experimental farm is located in coastal plains. All three fungal pathogens such as *Zasmidium* sp., *Passalora* sp. and *Pseudocercospora* sp. found to be associated with sooty blotch of bael fruits belongs to family *Mycosphaerellaceae* (*Capnodiales*, *Ascomycota*). In China, *Zasmidium litseae* has been reported to cause SBFS symptoms on the petiole of brown Bolly gum (*Litsea glutinosa*), based on phylogenetic relationship of ITS region and LSU loci along with morphological characterization (Zhao *et al.* 2016). *Zasmidium angulare*, as identified from the blotched fruit surface of *Malus domestica* in the USA causing SBFS based on morphology and phylogenetic analyses ITS and LSU loci. *Zasmidium* is presently placed in class Dothideomycetes, order Capnodiales (Hongsanan *et al.*, 2020). So far in Myco



**Fig 5. Scanning electron micrograph of sooty blotch fungi grown on bael fruit surface. The bar represents 50  $\mu$ m.**



**Fig 6. Impact of copper oxychloride (0.3 %) on sooty blotch fungal complex of bael fruit var CISH-B1 (a) Sprayed fruit (b) Unsprayed control fruit**

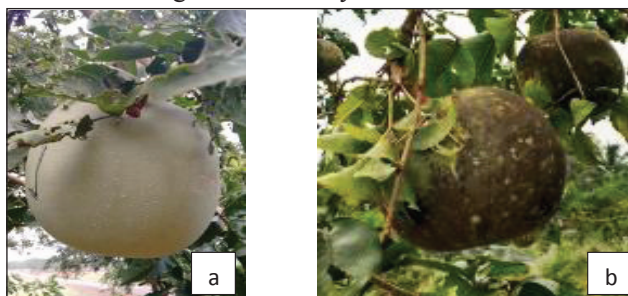
Bank 196 species of the *Zasmidium* genus (*Stenella*-like hyphomycetes) have been documented majorly from plant hosts (Crouset al. 2014). Similarly *Passalora*-like sp. FG3 and *Pseudocercospora* sp. LLS1 2 and *Pseudocercospora* sp. LLS2 has been documented to be associated sooty blotch of apple (Gleason *et al.* 2011).

The appearance of the blotch colonies was closely associated with the weather parameters. It is favoured by abundant rainfall, free water on fruit surfaces, high humidity with warm temperature. Since the growth of sooty blotch fungi was restricted to the fruit's surface, the nutritional requirements of the fungi must be available on the fruit's surface in order to sustain their growth. Earlier studies revealed that cuticular wax has its maximum influence on the regulation of cuticular permeability. Therefore, it is apprehended that the cuticle could play a role in determining the extent of growth of SBFS fungi by its permeability to nutrient (Belding *et al.* 2000). Further works are needed in this area on the permeability of the cuticle and fruit leachates in relation to sooty blotch severity. In a study with apple, Nasu and Kunoh (1987) observed that the flyspeck fungi *Zygothiala jamaicensis* did not penetrate cuticle, but when observed with scanning electron microscopy (SEM), waxy crystals appeared broken and dissolved along the hyphal strands. Mycelia of sooty blotch fungi, *Peltaster fructicola* grew on the waxy layer of apple, but did not seem to degrade it (Belding *et al.* 2000). In our study on growth of blotch fungi on bael fruits, the fungal growth was seen entirely on fruit's waxy layer and could not find evidence of disruption of waxy layer.

The blotch fungi survive on twigs of the bael tree may serve as an immediate source of inoculum for fruit infection evidenced from the greater sooty blotch colonies on the upper side of the fruits near to the peduncle in

addition to other reservoir hosts. In our study, we documented the sooty blotch fungal colonies on fruits as well as twigs of mango (*Mangifera indica*), Aou (*Dillenia indica*), Carambola (*Averrhoa carambola*), turkey berry (*Solanum torvum*) anola (*Phyllanthus emblica*) and twigs of jack fruit (*Artocarpus heterophyllus*), sapota (*Achras zapota*), star gooseberry (*Phyllanthus acidus*), acacia (*Acacia nilotica*), Simarouba (*Simarouba glauca*), piasala (*Pterocarpus marsupium*) and Calotropis (*Calotropis procera*). Layer of free water on surface of fruit is required essentially to initiate the infection. Sooty blotch infection may start at any stage of the fruit development if suitable environmental conditions are available. If favorable conditions continue, the fungal growth continues to cover almost 80-90 percent of the fruit surface and complete fruit becomes black. The study conducted in North Carolina, United States reported that the SBFS incidence reached 100% in the four locations selected for study by the last part of each growing season (Sutton and Sutton, 1994).

In our study, field spray of 0.3 percent copper oxychloride (first spray at the lemon stage, second and third spray at 15 and 30 days after the first spray, and the last spray at 30 days before harvest) provided around 96-97 percent blotch control. With the knowledge of the fact that the inoculum of sooty blotch-causing fungi is already present in the orchard at the time of fruit set, the management schedule needs to be implemented as a prophylactic way. In apple, control of sooty blotch and flyspeck was often achieved by timely fungicide sprays (Rosenberger *et al.* 1996). The fungicides used in this trial like thiophanate methyl, carbendazim and captan have been shown to control SBFS in apple orchards (Williamson and Sutton 2000). Factors like inefficient and poor fungicide coverage, weather conditions not allowing fungicide spray, and inaccessible tree height may lead to poor disease management. It is pertinent to emphasize the use of stickers and complete coverage of fruits by the fungicide solution are needed to achieve efficient management of sooty blotch.



**Fig 7. Effect of combined application of thiophanate methyl (0.2 %) + captan (0.1 %) on sooty blotch fungal complex of bael fruit var. NB-5. (a) Sprayed fruit; (b) Unsprayed control fruit**

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## REFERENCES

- Batzer, J. C., Gleason, A. J., Harrington, T. C., Mayfield, D. A. and Gleason, M. L. 2012. Temporal patterns in appearance of sooty blotch and flyspeck fungi on apples. *Microbial Ecology*, **64**(4): 928-941.
- Batzer, J. C., Gleason, M. L., Weldon, B., Dixon, P. M. and Nutter, J. R. F. W. 2002. Evaluation of postharvest removal of sooty blotch and flyspeck on apples using sodium hypochlorite, hydrogen peroxide with peroxyacetic acid, and soap. *Plant Disease*, **86** (12): 1325-1332.
- Batzer, J. C., Gleason, M., Harrington, T. and Tiffany, L. H. 2005. Expansion of the sooty blotch and flyspeck complex on apples based on analysis of ribosomal DNA gene sequences and morphology. *Mycologia*, **97** (6): 1268-1286.
- Batzer, J. C., Diaz-Arias, M. M., Harrington, T. C., Gleason, M. L., Groenewald, J. Z. and Crous, P. W. 2008. Four species of *Zygophiala* (Schizothyriaceae, Capnodiales) are associated with the sooty blotch and flyspeck complex on apple. *Mycologia*, **100**: 246-258.
- Belding, R. D., Sutton, T. B., Blankenship, S. M. and Young, E. 2000. Relationship between apple fruit epicuticular wax and growth of *Peltaster fructicola* and *Leptodontidium elatius*, two fungi that cause sooty blotch disease. *Plant Disease*, **84**: 767-772.
- Bensch, K., Braun, U., Groenewald, J. Z. and Crous, P. W. 2012. The genus *Cladosporium*. *Studies in Mycology*, **72**: 1-401.
- Braun, U., Crous, P. W., Schubert, K. and Shin, H. D. 2010. Some reallocation of *Stenella* species to *Zasmidium*. *Schlechtendalia*, **20**: 99-104.
- Brown, E. M. and Sutton, T. B. 1986. Control of sooty blotch and flyspeck of apple with captan, mancozeb, and mancozeb combined with dinocap in dilute and concentrate applications. *Plant Disease*, **70**: 281-284.
- Brown, E. M. and Sutton, T. B. 1995. An empirical model for predicting the first symptoms of sooty blotch and flyspeck of apples. *AGRIS*, **79**(11): 1165-1168.
- Crous, P. W., Lopez, G. A., Hawksworth, D. L., Robert, V., Kirk, P. M., Guarro, J., Robbertse, B., Schoch, C. L., Damm, U., Trakunyingcharoen, T. and Groenewald, J. Z. 2014. The genera of fungi: fixing the application of type species of generic names. *IMA Fungus*, **5**(1): 141-160.
- Dhakar, M. K., Das, B., Nath, V., Sakar, P. K. and Singh, A. K. 2019. Genotypic diversity for fruit characteristics in bael [*Aegle marmelos* (L.) Corr.] based on principal component analysis. *Genetic Resources and Crop Evolution*, **66**: 951-964.
- Diaz Arias, M. M., Batzer, J. C., Harrington, T. C., Wang, Wong., Bost, S. C., Cooley, D. R., Ellis, M. A., Hartman, J. R., Rosenberger, D. A., Sundin, G. W., Sutton, T. B., Travis, J. W., Wheeler, M. J., Yoder, K. S. and Gleason, M. L. 2010. Diversity and biogeography of sooty blotch and flyspeck fungi on apple in the eastern and midwestern United States. *Phytopathology*, **100**: 345-355.
- Doyle, J. J. and Doyle, J. L. 1990. Isolation of plant DNA from fresh tissue. *Focus*, **12**: 13-15.
- Dutta, A., Lal, N., Naaz, M., Ghosh, A. and Verma, R. 2014. Ethnological and Ethno-medicinal importance of *Aegle marmelos* (L.) Corr (Bael) among indigenous people of India. *American Journal of Ethnomedicine*, **5**: 290-312.
- Fries, E. M. 1849. *Summa vegetabilium Scandinaviae*. Sectio posterior, Uppsala.
- Gautam, A. K. 2014. Occurrence of black mildew on *Aegle marmelos* at Himachal Pradesh, India. *International Journal of Phytopathology*, **03** (03): 161-162.
- Gleason, M. A., Zhang, R., Batzer, J. C. and Sun, G. 2019. Stealth pathogens: the soot blotch and flyspeck fungal complex. *Annual Review of Phytopathology*, **57**: 135-164.
- Gleason, M. L., Batzer, J. C., Sun, G., Zhang, R., Diaz Arias, M. M., Sutton, T. B., Crous, P.W., Ivanovic, M., Mcmanus, P. S., Cooley, D. R., Mayr, U., Weber, R. W. S., Yoder, K. S., Ponte, E. M.D., Biggs, A. R. and Oertel, B. 2011. A new view of sooty blotch and flyspeck. *The American Phytopathological Society*, **95** (4): 368-383.



- Hemnani, K., Malley, P. J. O., Tanovic, B., Batzer, J. C. and Gleason, M. L. 2008. First report of seven species of sooty blotch and flyspeck on *Asimina triloba* in Iowa. *Plant Disease*, **92**: 1366.
- Hongsanan, S. et al. 2020. Refined families of Dothideomycetes: Dothideomycetidae and Pleosporomycetidae. *Mycosphere*, **11**(1): 1553–2107.
- Hosagoudar, V. B. 2011. The genus *Schiffnerula* in India. *Plant Pathology and Quarantine*, **1**(2): 131-204.
- Johnson, E. M., Sutton, T. B. and Hodges, C. S. 1997. Etiology of apple sooty blotch disease in North Carolina. *Phytopathology*, **87**: 88–95.
- Kamal. 2010. Cercosporoid Fungi of India. Bishen Singh Mahendra Pal Singh, India.
- Kamalakkannan, N. and Prince, P. S. M. 2003. Hypoglycemic effect of water extracts of *Aegle marmelos* fruits in streptozotocin diabetic rats. *Journal of Ethnopharmacology*, **87** (2-3): 207–210.
- Kintzios, S. E. 2006. Terrestrial plant-derived anticancer agents and plant species used in anticancer research. *Critical Reviews in Plant Sciences*, **25**(2): 79–113.
- Kumar, D. and Nath, V. 2010. Variability in bael (*Aegle marmelos* Correa) genotypes from Orissa. *Indian Journal of Plant Genetic Resources*, **23**(3): 303-305.
- Kumar, S., Stecher, G. and Tamura, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33**: 1870–1874.
- Li, H. Y., Sun, G. Y., Zhai, X. R., Batzer, J. C., Mayfield, D. A., Crous, P. W., Groenwald, J. Z. and Gleason, M. L. 2012. Dissoconiaceae associated with sooty blotch and flyspeck on fruits in China and the United States. *Persoonia. Molecular Phylogeny and Evolution of Fungi*, **28**: 113-125.
- Maurya, S., Kumar, R., Kumari, A. and Choudhary, J. S. 2016. First report of *Alternaria* leaf blight in bael (*Aegle marmelos* (L.) Corr.) from eastern plateau and hill region of India. *Journal of Agri Search*, **3**: 248-250.
- Mayr, U. and Spath, S. 2008. Sooty blotch of apple: Efficacy of different application strategies. In: Boos, Markus (Ed.) *Ecofruit - 13th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing: Proceedings to the Conference from 18th February to 20th February 2008 at Weinsberg/Germany*: 82-86.
- Mayfield, D. A., Karakaya, A., Batzer, J. C., Blaser, J. M. and Gleason, M. L. 2013. Diversity of sooty blotch and flyspeck fungi on apples in north-eastern Turkey. *European Journal of Plant Pathology*, **135** (4): 805-815.
- Mishra, A. K., Garg, N. and Yadav, K. K. 2016. First report of shell soft rot of bael (*Aegle marmelos*) Caused by *Syncephalastrum racemosum* in North India. *Plant Disease*, **8**: 1779.
- Mukherjee, P. K., Rai, S., Kumar, V., Mukherjee, K., Hylands, P. J. and Hider, R. C. 2007. Plants of Indian origin for drug discovery. *Expert Opinion on Drug Discovery*, **2**(5): 633–657.
- Nasu, H. and Kunoh, H. 1987. Scanning electron microscopy of flyspeck of apple, pear, Japanese persimmon, plum, Chinese quince and pawpaw. *Plant Disease*, **71**: 361-364.
- Patel, M. K., Allayya Navaramath, S. B. and Kulkarni, Y. S. 1953. Bacterial shot-hole and fruit canker of *Aegle marmelos* Correa. *Current Science*, **22** (07): 216–217.
- Patel, M. K., Bhatt, V. V. and Kulkarni, Y. S. 1951. Three new bacterial diseases of plants from Bombay. *Current Science*, **20**: 326-327.
- Pathirana, C. K., Rnaweera, L. T., Madhujith, T., Ketipearachchi, K. W., Gamlath, K. L. and Eeswara, J. P. 2020. Assessment of the elite accessions of bael [*Aegle marmelos* (L.) Corr.] in Sri Lanka based on morphometric, organoleptic, and phylogenetic relationships. *PLoS ONE*, **15**(5): e0233609.
- Quadevlieg, W., Binder, M., Groenwald, J. Z., Summerell, B. A., Carnegie, A. J., Burgess, T. I. and Crous, P. W. 2014. Introducing the consolidated species concept to resolve species in the Teratosphaeriaceae. *Persoonia*, **33**: 1-40.
- Rosenberger, D. A., Engle, C. A. and Meyer, F. W. 1996. Effects of management practices and fungicides on sooty blotch and flyspeck diseases and productivity of 'Liberty' apples. *Plant Disease*, **80**: 798-803.
- Rosenberger, D. A. and Meyer, F. M. 2007. Timing summer fungicides to control flyspeck disease

- on apples. *New York State Horticultural*, **15**: 10-13.
- Rosil, H., Mayfield, D. A., Batzer, J. C., Dixon, P. M., Zhang, W. and Gleason, M. L. 2017. Evaluating the performance of a relative humidity-based warning system for sooty blotch and flyspeck in Iowa. *Plant Disease*, **101**(10): 1721-1728.
- Shivas, R. G., Young, A. J. and McNeil, B. D. 2010. *Pseudocercospora microsori* Fungal Planet 68. *Persoonia*, **25**: 156-157.
- Sutton, A. L. and Sutton, T. B. 1994. The distribution of the mycelial types of *Gloeodes pomigena* on apples in North Carolina and their relationship to environmental conditions. *Plant Disease*, **78**: 668-73.
- White, T. J., Bruns, T., Lee, S. J. W. T., Taylor, J., Innis, M. A., Gelfand, D. H. and Sninsky, J. J. 1990. PCR Protocol: a guide to methods and applications. *Academic Press*, **38**: 315-322.
- Williamson, S. M. and Sutton, T. B. 2000. Sooty blotch and flyspeck of apple: Etiology, biology, and control. *Plant Disease*, **84** (7): 714-724.
- Xu, C., Chen, H., Gleason, M., Xu, J. R., Liu, H., Zhang, R. and Sun, G. 2016. *Peltaster fructicola* genome reveals evolution from an invasive phytopathogen to an ectophytic parasite. *Scientific Reports*. 6. 10.1038/srep22926.
- Zhao, W., Hou, Y., Bai, J., Zhang, W., Gao, L., Gleason, R. Z. M. L. and Sun, G. 2016. A new species of *Zasmidium* associated with sooty blotch and flyspeck. *Phytotaxa*, **258** (2): 190-194.

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