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G Shankar
Nichino India Private Limited, A-24/25, APIE, Balanagar, Hyderabad, Telangana, India

Corresponding Author:
G Shankar
Nichino India Private Limited, A-24/25, APIE, Balanagar, Hyderabad, Telangana, India

Report on the occurrence of summit disease syndrome in rice brown planthopper, *Nilaparvata lugens* (Stal) (Delphacidae: Homoptera)

G Shankar

Abstract
Naturally mycosed field population of the Rice Brown Planthopper (BPH), *Nilaparvata lugens* (Stal) was observed in a farmer's rice field in Andhra Pradesh. The adult insects exhibited typical *Summit Disease Syndrome* (SDS), which is considered to be a fungal-mediated behavioural change in the host. The infected insects, fully or partially covered with mycelial growth, were found firmly clinging to the tip of the leaves as solitary ones or in cohorts of 2, 3 or 4. They were immobile, either dead or in moribund state. Such insects, collected from the leaf tips along with live insects from the base of the plants, were examined in the laboratory. The preliminary microscopic observations showed the presence of micro and macroconidia which resembled *Fusarium* species. The resident Yeast-like symbiotes commonly present in healthy individuals were absent in live adults and nymphs of BPH collected from the infection site. Our present findings have contributed to an important knowledge in the area of insect pathogens and this could dislodge the notion that hyphomycetous fungi do not influence SDS in their hosts. The occurrence of Summit Disease transmissions in BPH suggests that this association must have been highly evolved.
These preliminary investigations are likely to arouse interest to look deep in to this host-pathogen relationship. Further research directions are suggested that may shed more light on the subject. This appears to be the first report on Summit disease syndrome in Rice brown planthopper.

Keywords: Rice brown planthopper, *Nilaparvata lugens,* summit disease, entomopathogenic fungus, *Fusarium,* yeast-like symbiote

Introduction

The Brown Planthopper (BPH), *Nilaparvata lugens* (Stal) is an important pest of rice, *Oryza sativa* L. (Poaceae) in many parts of India. The Southern states of India, more importantly Andhra Pradesh, are more affected by planthoppers, predominantly by BPH.

Farmers resort to chemical pesticides for managing BPH. However, natural infection of planthoppers is happening in rice fields, perhaps goes unnoticed by many. Quite a few entomopathogenic fungi [EPF] are reported to be occurring on BPH in nature.

One of the earliest reports of mycosis in BPH was the occurrence of white muscardine fungus [1]. Subsequently, existence of natural infection in BPH by *Pandora delphacis*, another entomophthoran fungus was reported [2, 3]. The Agriculture Research Station at Maruteru (Andhra Pradesh) has reported natural incidence of *Fusarium pallidoroseum* (Cooke) in field populations of BPH [4]. Natural mycosis of various growth stages of BPH by *Aspergillus spp, Rhizophus and Fusarium* was reported in rice fields of West Bengal [5].

The present report is a consequence of a serendipitous observation of mycosed population of BPH in a farmer's field in West Godavari in Andhra Pradesh. During one of the field visits to an experimental plot for evaluation of certain new generation biocides for the management of BPH, we were puzzled at the absence of adult hoppers at the base of rice plants in one replication of untreated control plot, while the rest of the control plots had good numbers. On a closer scrutiny we were surprised to see several adult hoppers clinging to the tips of leaves. They were in groups of 2 - 4 numbers and a few solitary hoppers were also observed at the tip of leaves. They were immobile and hence considered to be either dead or in a moribund state. In this investigation, we consider them as dead and refer to them as cadavers.

These serendipitous observations on the nature of the host-pathogen association are preliminary and largely qualitative.

Materials and Methods

Field Observations

The diseased Brown Planthopper cadavers were found in a private farmer's rice field in Suryaraopalem (coordinates: 16°48′12.2″N and 81°38′29.5″E) in West Godavari. The chance encounter happened on the 4th March 2020. Some initial observations were done on that occasion. After gathering certain basic knowledge on the subject further field observations were carried out on the 8th March 2020 and more relevant pictures were taken.

a) **Number of cadavers on leaf tips:** The number of cadavers on leaf tips was counted in 5.0 sq. m area within the 30.0 sq. m plot.
b) **Collection of insects:** The cadavers were collected together with the bits of leaf tips on which they were present and placed in a separate glass test tube. The live adults and nymphs were collected from the base of the plants and transferred to fresh stem pieces in another glass tube. There were very few live adults available at the base of the plant. The collected samples were transported to our laboratory at Masaipet in the state of Telangana for further observations.
c) **Field images of affected insects:** Appropriate images of the insects were captured using a 10X lens clipped to an *i-phone.*

Laboratory Observations

The field collected live insects from the base of the plants were segregated in to adults and nymphs. The adults were sexed and kept separately for observations. Similarly, the cadavers of male and female adults were also separated for observations.

Extent of Mycosis

a) External Observations

Certain individuals had abundant growth of fungus all over the body, quite visible to the naked eyes, while others did not show any external indication of fungal presence. Nevertheless, the insects were individually observed with a handheld lens to check the extent of fungal growth on the body surfaces and other appendages.

b) Microscopic Observations of Crushed Bodies

The samples were individually crushed on glass slides using a few drops of double distilled water to observe the internal contents. The slides mounted with coverslips were observed under OLYMPUS CH 20i light microscope at 40x magnification. The conidia of pathogen from the field collected cohorts of BPH were observed qualitatively for their abundance, for a preliminary understanding.

Presence of yeast-like symbiotes in cohorts of field collected samples and laboratory reared BPH was qualitatively assessed and compared.

Micrographs were taken using i-phone fitted with a 10 X clip-on lens, with the resulting images of 400X magnification.

Fig 1: Summit Disease Syndrome in mycosed BPH adult. Arrows show dark and hyaline spores at the tip

Fig 2: Tuft of fungal structures arising from ventral side of abdomen [arrow]. Wings are outstretched

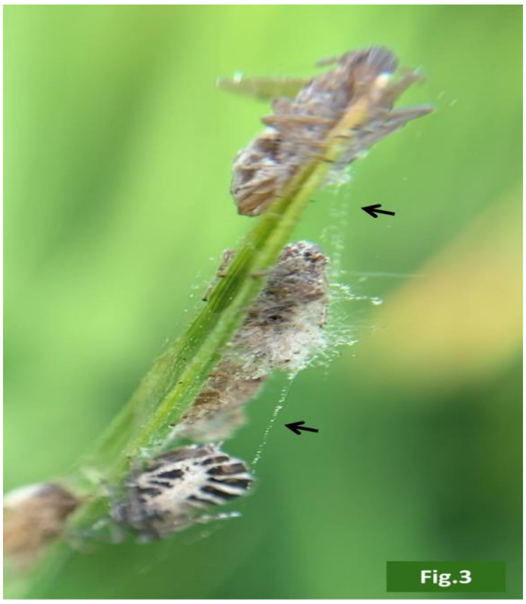

Fig 3: Individuals in a cohort are interconnected with mycelial strands [shown by arrows]. The insect at the bottom has melanized cuticle.

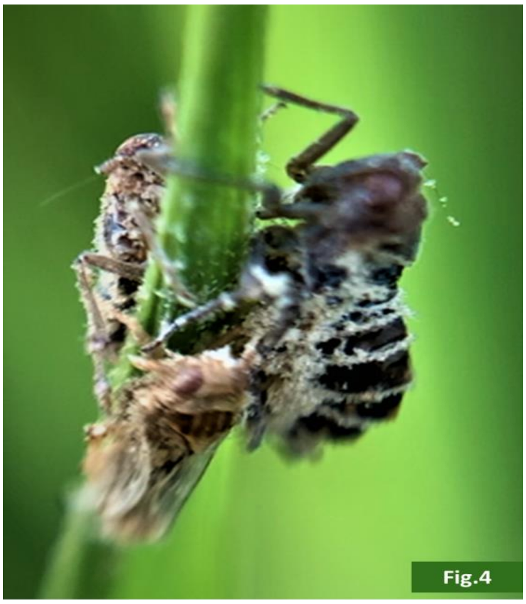

Fig 4: The fungal growth seen on antenna, leg joints, spiracles and integumental segment. The body is melanized.

Results

Field observations

The number of cadavers clinging to the leaf tips was 86 in 5.0 sq. m area in the centre of the 30.0 sq. m plot. The observations revealed the presence of cohorts of 2, 3 or 4 insects per leaf. There were very few solitary insects. None of the insects present at the tip appeared to be alive. Most of them did not respond to prodding. They must have been either dead or in a state of morbidity.

Most insects had profuse, whitish mycelial growth covering their entire body from all sides [Fig. 1]. On pressing between fingers some of these individuals have crumpled indicating that the internal contents have been fully utilised by the fungus. Individuals without any visible external growth and with their outstretched wings appeared to be recently died. A few insects had a small tuft of fungal growth arising from their soft bellies [Fig.2]. Their body contents appeared to be intact and squeezed out on applying slight pressure.

The solitary insects or those in cohorts which reached the summit first were present almost closer to the leaf tip, just 0.5-1.0 cm below the tip [Figs.1, 2, 3 and 10]. They were holding the leaf tip tightly with 2 or all 3 pairs of legs. In some insects the first pair of legs was free and out stretched. In cohorts, the top most insect was closer to the tip and the others were 1-3 cm away from the first insect, but were huddling closer to each other. The fungal strands arising from one cadaver connected all neighbouring individuals [Fig-3].

In some individuals the whitish fungal growth could be seen on antenna, spiracles, between body segments, and leg joints [Fig-4]. A few insects appeared to have highly melanised abdomen [Fig 4, 5 and 6].

No mycosed nymphs of BPH were found on the leaf tips.

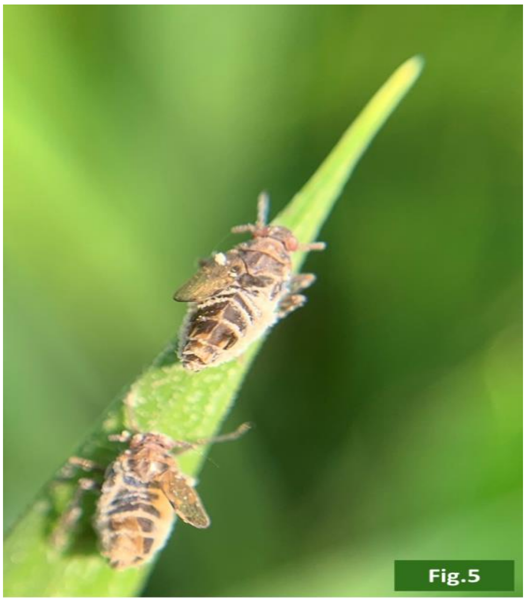

Fig 5: Mycosed insects with melanised cuticle. These Brachypterous females have outstretched wings. The insect at the top is clinging to the leaf at the narrow tip of the leaf. The insect next to that is managing to hold the leaf may be bound by some fungal substance.

Fig 6: Melanised body. Outstretched wings. Fungal growth emerging between body segments

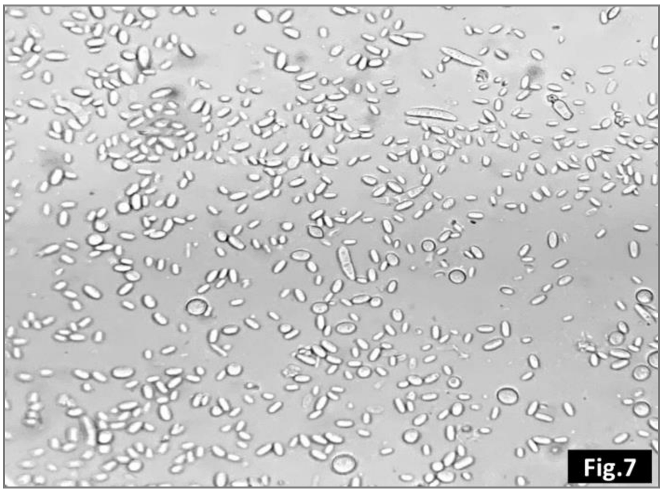

Fig 7: Abundant presence of Microconidia in the field populations of infected BPH cadavers. They are oval in shape. A few are globose shaped. 400X

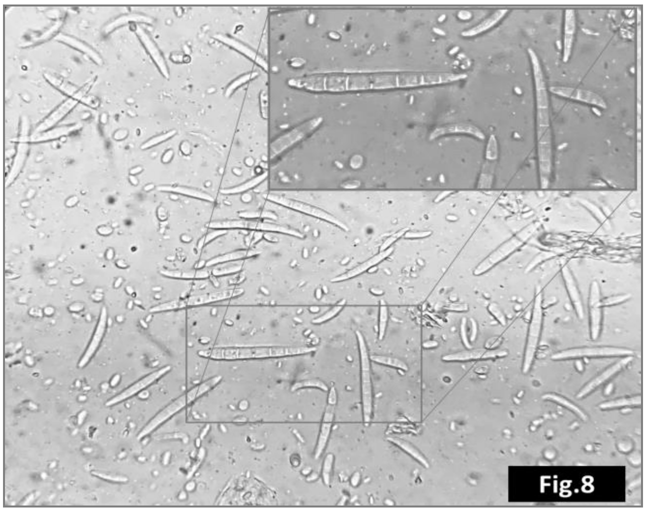

Fig 8: Large number of Septate Macroconidia [portion magnified in inset] present in the cadavers of BPH collected from the field. 400X

The live insects at the base of the plants had a mixed population of adults and nymphs. The adults were fewer and rare to be seen at the base, on the day of observation. They were slow in response to prodding. On the other hand the nymphal stages were active.

The summary of results of external observations is presented in Table-1.

Table 1: Summary of results of external observations of field collected cadavers & live insects

Status	Observations
Dead insects	• 39 female insects have exhibited full bloom to a little external growth on the surface. • In some individuals the whitish fungal growth could be seen on antenna, spiracles, between body segments, and leg joints. • A few insects appeared to have highly melanised abdomen.
Dead insects	• 25 female insects had no visible growth on the dorsal surface. In a few the ventral side of abdomen exhibited small tuft of fungal filaments. • Of them, 6 females had their wings outstretched exposing the dorsal surface of abdomen.
Dead insects	• 8 male insects had no visible external growth of fungus.
Live insects	• The live nymphs were active and did not have any visible fungal growth on the external body surfaces.
Live insects	• The live adults were very few and were inactive. No visible external growth of fungus. Exhibited lazy response to prodding.

Laboratory observations

Out of 72 cadavers transported to the laboratory 64 were adult females and 8 were adult male BPH. No nymph was found among them. The details of observation are provided in Table-2.

From the scrapings of external fungal growth and crushed bodies of dead cadavers collected from the leaf tips of rice plants abundance of micro and macroconidial structures could be observed [Fig. 7 & 8]. Contrarily, the cadavers exhibiting only slight fungal growth on the abdominal intersegmental areas had a large number of microconidia. Macroconidia were very few.

Similarly, male cadavers also exhibited larger population of microconidia.

The Live female adults collected from the base of the plants had a fewer micro and macroconidia while the live older nymphs did not possess any conidial structure inside their bodies.

However, only some of the older nymphs possessed a few cells of yeast like symbiotes in the body crushing. None of the remaining samples had their symbiotes.

The laboratory reared healthy female adults exhibited a huge number of cells of symbiotes [Fig.9]. There was no presence of conidial structures in these healthy individuals.

Conidial structures

The microconidia and macroconidial structures were observed in large numbers in the cadavers collected from the field. The microconidia were largely oval shaped; a few were globose shaped [Fig.7].

The macroconidia were long and mostly tapering in one end, while the base appeared wide and pedicellate. Though largely appeared straight, a few were slightly curved at the tapering end. The cells were septate and mostly had up to six walls [Fig.8], while a few showed 3-4 septa.

Table 2: Summary of observations of crushed samples

Sl. No	Collection Position	Number	Fungal growth status	Development Stage	Sex	Microconidia	Macroconidia	Yeast-Like symbiotes
Samples collected from untreated control plot in the field								
1	Leaf Tips	39	Cadavers with external growth	Adults	Female	Large numbers	Large numbers	Absent
2	Leaf Tips	25	Cadavers – a few had fungal growth on the ventral abdomen and some in intersegmental areas	Adults	Female	Large numbers	Fewer	Absent
3	Leaf Tips	8	Cadavers – no visible growth	Adults	Male	Large numbers	Fewer	Absent
4	Base of plant	14	Live – no growth	Adults	Female	Fewer	Almost absent	Absent
5	Base of plant	39	Live – no growth	Older nymphs	Mixed	Absent	Absent	Nil - Very few
Live healthy adults from laboratory culture								
6	Base of plant	5	Live	Adults	Female	Absent	Absent	Large numbers

Discussion

Microscopic observations of body scrapings and crushed insects:

The microscopic observations of the live and dead insects have been summarised in Table 2. The conidia of EPF largely resembled that of *Fusarium*. It will be confirmed only after isolation.

Fusarium species were, in general, believed to be a significantly important group of plant pathogens. A recent review has enlisted seven *Fusarium* spp., exhibiting entomopathogenicity. They had been isolated from the soils from forests, fruits and vegetable orchards in China and Palestine. They were *Fusarium avenaceum* (Fr.) Sacc., *Fusarium heterosporum* Nees, *F. moniliforme*, *F. oxysporum*, *F. semitectum* Berk & Ravenel, *F. solani* and *F. redolens* Wollenw [6]

There have been reports on the presence of *Fusarium* spp associated with the infected cadavers of the rice brown planthopper [4, 5].

From the summary of our observations in Table 2, it can be seen that abundance of micro and macroconidia differed in live and dead individuals and also between live adults and nymphs.

The absence of any conidial structures in the live nymphs may mislead one to believe they were healthy. The nymphs were perhaps in their early phase of infection. May be a detailed histological study is required to get more answers. However, the pathological condition of the nymphs could be well understood from the status of their hereditary symbiotes, which were either totally absent or only a few were present.

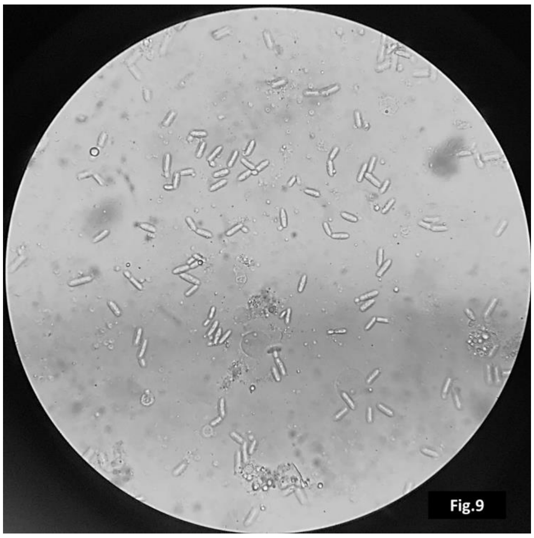

Fig 9: Cells of intracellular yeast-like symbiotes present in laboratory reared healthy BPH adults. 400X

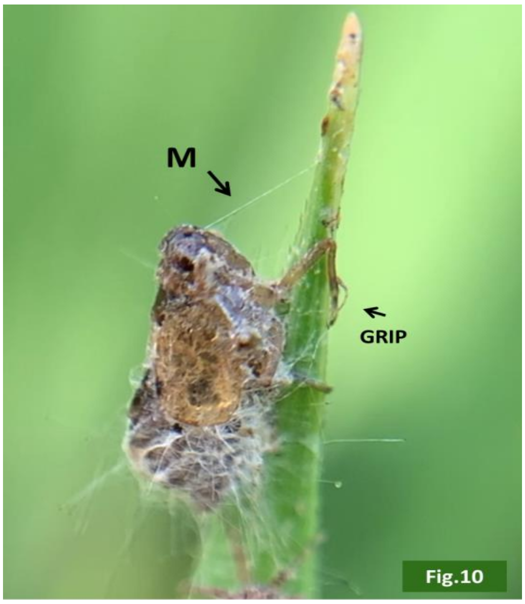

Fig 10: A mycosed adult at the tip of the leaf. Notice the tight grip using all pairs of legs. Position of abdomen is closer to the substrate. Fungus ensures host is secured to leaf by binding with mycelial strands [M] to leaf.

Fig 11: Summit disease exhibited by typical grip with all pairs of legs. Notice the position of abdomen close to the leaf. This is the unusual position of the planthopper

A similar observation in field collected live adults revealed the presence of only a few conidia while symbiotes were totally absent, confirming the existence of a similar pathological condition in them too as described for live nymphs. This could be a quick test to confirm 'that all is not well' with the insect.

The abundance of microconidia in cadavers with slight external growth of fungus indicates that macroconidia was perhaps the next growth stage of the fungus, just before dissemination.

The large numbers of both forms of conidia in 39 cadavers that have exhibited full growth of fungus on the body indicate that the fungus has completely developed and were ready for dispersal. The presence of macroconidia in them perhaps suggests they could be the infective stage for the perpetuation of this fungus infecting BPH.

Some researchers while describing the *Fusarium* infection in *G. mellonella* larvae opined that conidial morphology played a vital role in infection and reported that macroconidia were more virulent than the microconidia in *Fusarium* [7].

Process of infection & dissemination – a possible dual approach by the pathogen

The data that we have from the field is inadequate to determine the possible infection process. It requires a systematic laboratory investigation. However, our data points to a possibility that the very presence of live infected nymphs and adults at the base of the plants is an indication that Primary infection and dissemination are happening at the site of host's habitat, where normally a large population lives. Earlier researchers have collected infected BPH nymphs and adults, with visible fungal growth from the body, from soils in West Bengal rice fields [5]. Besides several EPF, they have isolated *Fusarium* also as one of the pathogens from those cadavers. Another team of researchers from Andhra Pradesh have isolated *Fusarium pallidoroseum* from infected cadavers of BPH collected from the base of rice tillers in the field [4]. Fusarium's ability to survive in soil for longer periods strengthens the possibilities of their natural insect-pathogenicity in fields [6]. This confirms our suggestion that the primary infection process is happening at soil level in the rice fields.

In addition to the above, through a Secondary process, the pathogen appears to have ensured an efficient dissemination of infection bodies far and wide by inducing the infected BPH adults to climb up to the Summit.

Impact on the intracellular symbiotes

The examination of crushed samples of live nymphs and adults collected from the infection site revealed the absence of yeast-like symbiotes. Normally a healthy adult female BPH carries around 50^5 yeast-like symbiotes in its abdominal fat bodies [8]. The symbiotes in rice planthoppers are nutritionally important to their hosts as they reportedly synthesised and offered the dietary sterol to the host. The host insect is neither capable of synthesising sterols by itself nor it is a component of phloem sap in rice plants. When these symbiotes were experimentally eliminated, the resulting non-availability of sterol to the hosts adversely affected their normal development and reproduction [9]. Available evidences suggest that 'stressed' animals are more susceptible to entomopathogens than non-stressed ones [10, 11]. The nutritional stress could be a factor in determining the susceptibility of the insects to entomopathogens.

In the present observations, considering the presence of large number of symbiotes in the laboratory reared healthy adults and the disappearance of symbiotes from the field collected EPF-infected live BPH nymphs and adults lead us to a possibility of EPF exerting adverse impact on the symbiotes. One possibility could be the release of some kind of toxins by the invading pathogen during the process of penetration of the host cuticle. Certain EPF like *Fusarium* are reported to produce well known fungi-toxins like beauvericin and enniatins [12]. May be the loss of symbiotes will weaken the BPH to render them more susceptible to the fungal invasion.

In case of *Philanthus triangulum*, its symbiont *Streptomyces* spp produced nine kinds of antibiotics to protect the host larvae against certain fungi and bacteria [13]. In *Blatella germanica* their symbiotic gut flora helps in resisting the infection by fungi [14]. No information is available on the role of BPH symbiotes in safeguarding their host insect from invading pathogens. Such studies are required to understand the interaction of symbiotes and EPF in BPH.

Summit disease syndrome (SDS)

The interaction of EPF with its host insect begins with the infection and multiplication inside the body of the host. This is the routine, classic process of parasitisation. Interestingly, in some cases the pathogen goes beyond this familiar process, making the association more complex by taking complete control of the host's behaviour.

One of the earliest reviews on hypomycetous fungi as EPF described that grasshoppers infected with *Entomophaga grylli* often climbed to the tree tops before the death [15]. This behaviour was termed as Summit disease syndrome (SDS). A recent report provided a detailed account of occurrence of SDS in 28 species of grasshoppers in Kazakhstan [16].

It is said the pathogen manipulates the behaviour of host to its advantage, particularly to achieve high degree of dispersal for infecting fresh individuals of its hosts. This behavioural change in insects often determines the infection reaching epizootic magnitudes. Presence of SDS is considered as a high level of adaptation of the pathogen to the host, as it keeps the host under its control and alive till the insect reached a desired height [17].

In the present investigation, the presence of large number of mycosed cadavers of BPH adults near the leaf tips confirms the existence of SDS in the rice brown planthopper, which no one seems to have observed or reported so far. Therefore, we believe our observations are the first of its kind in the rice brown planthopper.

Another observation of interest in the present study was wherever clusters of 3-4 cadavers were present; there every individual was connected with the fungal structures [Fig.3]. This may be a strategy of the fungus to infect every individual host in the vicinity to maximize the chances of effective self-perpetuation.

Further, we believe, that our observation of the outstretched wings of both Macropterous and Brachypterous BPH cadavers [Fig 2, 5 and 6] could be yet another fungus-mediated behaviour. The normal position of the planthopper wings, covering a major portion of host's dorsal abdominal surface, could be a barrier for the emergence of fungus. So the fungus cleverly manipulates the host to spread its wings to facilitate uninterrupted emergence and growth of fungal structures from haemocoel. It is believed that SDS in both Entomophthoromycota and Ascomycota is so evolved that they induced their hosts to have the wings outstretched to allow spores to be discharged from the body [18]. In some of our cadaver samples the location from where the fungus first emerged seemed to be ventral abdominal portion [Fig.2]. In case of infection of brown planthopper by *Pandora* sp too the first place of mycelial emergence was reported to be from abdomen of the dying host [19].

There are references of 'Death Grip' where the affected host after climbing to the top holds the plants tightly and bites into the leaves [18]. This could be yet another fungus-mediated behaviour to prevent possible fall of the hosts after their death. In the present study it was observed that all the adult planthoppers located at the tip of leaves found to have clung to the leaves with either two or all three pairs of legs [Fig.10, 11]. The hoppers have positioned their bodies more closely to the leaf surface, so that they could hold the leaf tightly. In normal field and laboratory adults there is always a clearance between their ventral abdomen and the substrate on which they rest.

The rice leaf is generally 15-20 mm broad and gradually tapers upwards to measure 2-3 mm towards the tip. The possible reason for the insects traveling all the way to the tip of the leaves [Fig.1, 2 and 3] is to get into correct position and right grip before death arrived. Nevertheless, the pathogen doubly ensures cadaver's security by binding them to the leaves by the fungal structures [Fig.10]. Then the question arises, how the other insects following the first one are secured to the leaf. These insects are positioned on a broader plane where they cannot get any good grip to hold on to the substrate. It is assumed that they are likely to have been bound by the fungal strands. This needs to be verified. Though the present case is not similar to the reported death grip in ants where the mandibles are pushed deep into the leaf veins, it would be interesting to study how the planthopper stylets are positioned. Do the stylets remain free or inserted deep into the mid-ribs of the leaves to anchor themselves from falling?

The occurrence of melanised individuals is a common sight in immigrant populations, mainly in macropterous individuals. But the significance of presence of more melanised brachypterous adults at the summit is unclear.

The presence of a few male cadavers at the leaf tips, however small in numbers, points to the fact that the fungus does not discriminate the sex of the host. It effectively uses both the sexes to its advantage.

The Indian researchers who have documented the occurrence of EPF in rice brown planthopper have not reported the phenomenon of SDS [1-5]. They have collected the mycosed cadavers of brown planthoppers from the base of the rice hills [4] or from the soil surface in rice fields [5]. Some researchers believe that SDS may not be occurring in insects infected with hyphomycetous fungi [15]. In this context our present findings have contributed to an important knowledge in the area of insect pathogens and this could dislodge the notion that hyphomycetous fungi do not influence SDS in their hosts.

Recently, different modes of transmission adopted by EPF has been categorised as Active Host Transmission [AHT], Summit Disease Transmission [SDT] and Non-Manipulative Transmission [NMT] [20]. In AHT the fungus not only manipulates the host's behaviour but grows and produces the infectious spores in live insects, keeping the insect functional till the transmission is achieved [as seen with *Massospora* on Cicadas]. With SDT the host is again influenced by the fungus but kept alive only till the host reaches a convenient position on the plant canopy [example *Entomophaga, Ophiocordyceps* on grasshoppers and ants]. Here the fungus grows on dead or moribund hosts and later spores are released. NMT happens mostly passively through environment and by contact of infected host with other healthy individuals [as in *Metarhizium and Beauvaria* on planthoppers].

Based on our current observations we can extend the above categorisation to rice brown planthopper to say that both NMT and SDT are operational as two unique modes of transmission of EPF. While NMT could be conventional in BPH infected by *Metarhizium, Pandora* and *Beauvaria,* the SDT, now reported in the present study, may be considered as more evolved method of transmission in rice brown planthopper.

Next course of work

The present work is largely preliminary and qualitative in nature and the observations on Summit Disease Syndrome in Rice Brown Planthopper have been related to the available descriptions of SDS in other insect species. Much remains to be understood on the identity and nature of the EPF involved. Some research directions are put forth here that may shed light on this interesting host-pathogen relationship.

a) Isolation and identification of EPF reported in the present study to be completed. Koch's postulates for pathogenicity to be confirmed.
b) The process of infection in nymphs and adults, from beginning to '*End Game*' stage, is to be studied through histological observations.
c) Tracing the biochemical changes in infected hosts from beginning to end
d) The SDS is to be studied in detail in simulated laboratory / Greenhouse experiments.

Conclusion

From the foregoing results and discussions it may be concluded that this work had brought to the fore the occurrence of Summit Disease Syndrome in rice brown planthopper, which has not been known so far. This is believed to be a fungus-mediated behaviour in the host insects. The infected individuals, irrespective of their development stage have lost their hereditary yeast-like symbiotes. The EPF involved is suspected to be a *Fusarium* sp. This appears to be the first report on summit disease in BPH.

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