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Natural Mycosis of Rice Brown Plant Hopper (*Nilaparvata lugens* Stål) in Eastern India

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Abstract

The studies on natural mycosis of brown planthopper(BPH) in rice ecosystem of Eastern India found that *Aspergillus flavus* Linkk. caused maximum mortality (20 to 30%) followed by *Aspergillus niger* (Teig.) (15 to 20%), *Rhizopus* sp. (10-20%)and *Fusarium* sp. (5-20%) . In some cases more than one fungus was found to colonise the dead insect viz. (*Rhizopus* + *Trichoderma*) - 5 to 15%, [*Aspegillus flavus* Linkk. + *Trichoderma* sp.] – 10%, [*Fusarium* sp. + *Trichoderma* sp. + *Aspergillus flavus* Linkk.]-5 to 10%. Most of the fungi infected the insect solely but only the *Trichoderma* sp was not found as sole coloniser of the BPH. The data also showed that natural mycosis were most effective in I and II instars (0-25% BPH colonised) followed by III instar (0 to 20% BPH colonised), Vth instar (10 to 15% BPH colonised), IV instar (0 to 15% BPH colonised) and winged adult (5 to 10% BPH colonised). Association of multiple fungus species with the dead insect was low in early stages (I, II and III instar) rather than IV, V and adult. Generally incidence of *Aspergillus flavus* Linkk on BPH appeared during the second week of September to third week of November with two distinct peaks on 3rd week of September and 4th week of October respectively. The correlation studies in 2 different agro- climatic zones showed that the BPH population had a significantly negative correlation with the percent infection by fungi. . The effect of different formulations of this natural Mycosis in laboratory showed that % mortality was highest (16%) in Talk based followed by wettable powder of China clay (10.60%) and were significantly higher than water suspension (8.05%). The interaction between two factors i.e. spore (A) x base (B) was also statistically significant.

Keywords: Fungus; Brown planthopper; Mycosis; Cadavers; Homoptera.

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1. Introduction

Brown plant hopper (BPH) *Nilaparvata lugens* Stål. belonging to the order Homoptera is a serious pest causing damage to rice. Though this insect is known to occur in Asia since late forties, it was earlier only a minor pest of rice [1]. During the 1970s and the early 1980s the pest posed tremendous problems and was referred to as "a threat to Asian rice production. Since then BPH had damaged the rice crop in Malaysia, Philippines, Thailand & Bangladesh causing enormous losses in rice production [2].

The detail studies on natural mycosis started in India at the end of twentieth century when [3] studied the occurrence of a fungal disease on brown plant hopper of rice and found that the nymphs and adult BPH were died in rearing cage due to infection of *Fusarium moniliforme* var. intermedium. Among the other workers [4] reported that *Fusarium* sp. and *Verticillium* were the entomopathogenic fungi as important natural enemies of BPH from India. Number of workers have studied in this field mention may be made [5-16].

2. Materials and Methods

A survey was conducted to find out the natural mycosis of brown plant hopper *Nilaparvata lugens* Stål. in four different localities under two different agroclimatic zones viz. Red and Laterite zone and New Alluvial Zones of West Bengal. The first two localities (Bankura and Birbhum)under Red and Laterite zone were located between 20 °31´ and 27°14´ North latitude and 85°53´ East longitude and other two localities (Nadia and Burdwan) between 22°50´ North latitude and 88°21´ East longitude.

Dead or mummified BPH with fungal outgrowth were found lying on soil surface under the plant were collected and washed with tap water to remove excess waste materials. The infected BPH were planted on modified Sabouraud's Agar medium with antibiotic compounds chloramphenicol 100 ppm and sodium taurocholate. Plates were incubated at 30^0C temperature and 90% relative humidity at laboratory in the Department of Plant Pathology, BCKV, Kalyani India . Consequently another experiment was conducted under laboratory conditions with mixed fungal species consortia which was obtained by crushing the fungal infected BPH from the fields. The crushed materials were spread over both talk powder and wettable powder of China clay and the spores were counted under microscope for finding the strength of the formulated product . The efficacy of the different formulations of this natural fungi were evaluated against brown planthopper in laboratory at Regional Research Sub Station, BCKV, Chakdaha, WB, India.during 2014-15 and 2015-16

3. Results Discussions

From the laboratory study it was found that the dead brown planthopper samples collected from two different agroclimatic zones of WestBengal in Eastern India were infected by *Aspergillus flavus* (Link), *Aspergillus niger* (Teig), *Rhizopus* sp, *Fusarium* sp, [*Rhizopus* sp. + *Trichoderma* sp.], [*Aspergillus flavus* (Link) + *Trichoderma* sp] and [*Fusarium* sp + *Trichoderma* sp + *Aspegillus flavus* (Link)] as given in Table 1.

Table 1: comparitive development of fungal species of BPH in two different agro climatic zones of west Bengal

Sl. No.	Fungi	Percent BPH nymph (III + IV + V instar) colonized in			
		Red and laterite zone		New Alluvial Zone	
		(Bankura)	(Birbhum)	(Nadia)	Burdwan
1.	*Aspergillus flavus* (Link.)	20	25	30	25
2.	*Aspergillus niger* (Teig.)	20	15	15	15
3.	*Rhizopus* sp.	0	20	10	15
4.	*Fusarium* sp.	5	20	10	20
5.	*Rhizopus+Trichoderma* sp.	0	5	15	15
6.	*Aspergillus flavus*(Link.) + *Trichoderma* sp.	0	10	10	5
7.	*Fusorium* sp. + *Trichoderma* sp. + *Aspergillus flavus*(Link.)	0	5	10	5

Values (Mean of 20 BPH nymph (III + IV + V) from each location during end of October to first week of November.

The study also showed that the cadavers of the BPH were affixed to the culm of the rice plants and the fungus isolated from these sample proved its pathogenicity. Of the five species *Aspergillus flavus* (Link) caused maximum mortality 20 to 30% followed by, *Aspergillus niger* (Teig) 15 to 20%, *Rhizopus* sp 10 to20% and *Fusarium* sp (5 to 20 %) respectively. In some samples more than one fungus was found to colonise the dead insects where *Rhizopus* sp + *Trichoderma* showed maximum mortality 5 to 15%, followed by *Aspergillus flavus* (Link) + *Trichoderma* sp – 10%;*Fusarium* sp + *Trichoderma* sp + *Aspergillus flavus* (Link) 5 to 10% respectively . Although the *Trichoderma* sp was not found as sole coloniser of the BPH but it can suppress the growth of other fungal population in laboratory. Reference [6] reported that nymphs of brown plant hopper of rice *Nilaparvata lugens* Stål. were more susceptible to attack by *Fusarium oxysporium*ain India and later [7] also reported mortality of BPH population by *Fusarium oxysporium* in China. Reference [17] isolated *Aspergillus flavus* (Link), *A. niger*, *Rhizopus* sp., *Fusarium oxysporium* from BPH in India. Reference [18] recorded mortality of BPH due to *Fusarium moniliformis* var intermedium in the Southern part of India. The authors also found mycelium of the fungus emerged through intersegment membranes of abdomen and the joints of legs. The network of the white mycelium developed all over the body. The insects were found either sticking to leaf sheath or floating on standing water, being overgrown by white mass of spores. In the present investigation the BPH infected with *Aspergillus flavus* (Link) showed swelling of the thoracic region and crumbling leg. Reference [19] reported that *Rhizopus oryzae*, *Fusarium* sp were pathogens of BPH infecting all instars and forms of the adult hopper. Reference [20] studied the interaction of entomopathogenic fungus *Fusarium pallidoroseum* (Cook) Sacc with *Trichoderma* sp. and found that the growth of *F. pollidoroseum* was inhibited significantly by the *Trichoderma* sp. The zone wise survey on natural mycosis in eastern India indicated that among the four different localities the highest % of mortality was recorded in New alluvial Zones followed by Red and Laterite Zones of WestBengal. All the 5 different fungal species were recorded in Nadia , Burdwan and Birbhum but the BPH cadavers in Bankura harbors only *Aspergillus* sp. and *Fusarium* sp as given Table-1

Occurrence of natural fungus on different stages of brown plant hopper

The study was undertaken to find out the occurrence of colonizing fungi on different nymphal instars of BPH in rice crops. Dead BPH having distinctly colonised by fungus were collected from rice fields of Nadia and brought to the laboratory for isolation of fungi in modified sabouraud's Agar medium with antibacterial antibiotic (chloramphenical 100 ppm and sodium taurcholate). The fungi found to be associated with different stages of BPH were composed of same species as observed in the earlier study (Table 2).

Table 2: Occurrence of indigenous fungi on different stages of Brown Plant Hopper

Sl. No.	**Fungi in association with BPH**	**Percent BPH colonized**					
		I + II instar nymph	**III instar nymph**	**IV instar nymph**	**V instar nymph**	**Adult (winged form)**	**Total**
1.	*Aspergillus flavus* (Link)	25	20	15	15	15	85
2.	*Aspergillus niger* Teig	20	15	10	10	10	65
3.	*Rhizopus* sp.	20	10	10	10	10	60
4.	*Fusarium* sp.	20	20	15	10	5	70
5.	*Rhizopus* sp. + *Trichoderma* sp.	10	10	0	10	5	35
6.	*Aspergillus flavus* + *Trichoderma* sp.	15	10	10	15	10	60
7.	*Fusarium* sp. + *Trichoderma* + *Aspergillus flavus*	0	10	8	10	5	33
	Total	95	95	70	80	60	

Values are average of insects at each stage. I & II nymphal stages were observed on 13.10; 05, IV & V nymphal stages and adult BPH were observed on 24.10.15.

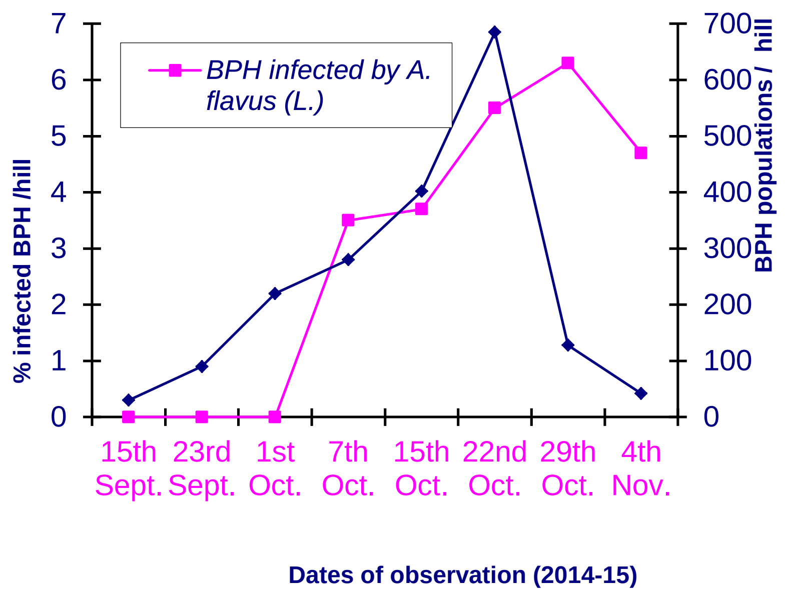
The data presented in Table 2 indicated that BPH was more susceptible to attack by *Aspergillus flavus* (Link.) (15 to 25%) followed by *A. niger* Teig (10 to 20%), Rhizopus sp. (10-20%),and *Fusarium* sp. (5 to 20%) . On the basis of the colonization of fungus on BPH the order of the susceptibility in descending order were., [*Aspergillus flavus* (Link) + *Trichoderma* sp.], [*Rhizopus* sp. + *Trichoderma* sp.] and [*Fusarium* sp. + *A. flavus* (Link) + *Trichoderma* sp.] with their percent BPH colonized (10 to 15%), (0 to 10%), (0 to 10%), respectively. [7] reported 51-80% mortality of BPH population by *Fusarium oxysporium*. Reference [18] recorded that average mortality of BPH was 8.20% with a range of 4.80 to 15.80% due to *Fusarium moniliformis* var intermedium during the cooler months of the year. A study conducted by in vitro dual culture technique on the interaction of *Fusarium pollidoroseum* with *Trichoderma* revealed that the latter one exerted 60.97% inhibition of *F. pollidoroseum* in the laboratory [20]. The relative susceptible values of indegenous fungus were compared with those of different stages of BPH reported by [6]. The data presented in Table 2 showed that most of the natural mycosis were most effective in I and II instars (0-25% BPH colonised) followed by III instar (10 to 20% BPH colonised), Vth instar (10 to 15% BPH colonised), IV instar (0 to 15% BPH colonised) and winged adult (5 to 15% BPH colonised). Association of multiple fungus species with the dead insect was low in early stages (I, II and III instar) rather than IV, V and adult. *Trichoderma* sp. although found to be associated as one of the natural mycosis with BPH, it was never found to be colonised as single species observed in the present investigation.

Influence of some abiotic factors (temperature and relative humidity) on the pathogenicity of *Aspergillus flavus* (Link.) to BPH in the plains of West Bengal

Abiotic factors like temperature and humidity play important roles on the pathogenicity of *Aspergillus flavus* (Link) to the BPH in rice fields. The observations were recorded from the symptoms of infected BPH such as swelling of thoracic region, becoming sluggish, crumbling legs followed by death of the insect.

A field study was conducted in three different villages Bankura; Birbhum; andNadia and the data are presented in Table 3. It is evident from study the pathogenicity started during the first week of October and peaked on the at 3rd week of October followed by sudden decline after 2nd week of November whereas in the district of Birbhum the fungus appeared during the last week of September but maximum infection occurred at 3rd week of October and disappeared after second week of November.

In Nadia the pathogenicity of BPH appeared early in the 3rd week of September but high intensity of infection appeared during 2nd week November.



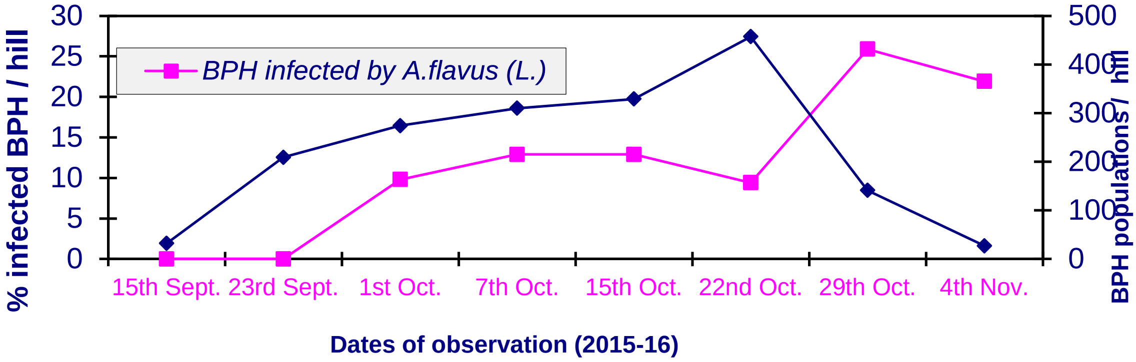


Figure 1: Seasonal incidence of BPH and the population infected by *Aspergillus flavus* (L.) in Eastern India during 2013-2016

Correlation studies showed that both maximum relative humidity (98%) and minimum temperature (21^0C) at Bankura had significant positive correlation with percent of pathogenicity whereas minimum relative humidity maximum temperature showed significantly negative correlations with the fungus infection. The correlation studies at Birbhum and Nadia also showed the identical results obtained from Bankura as given in Table 3. Reference [21] reported that fungal pathogen flourish abundantly in the rice ecosystem with its prevailing high humidity and infection occur naturally.

The author also reported that entomopathogenic fungi infect the host through the cuticle. The process of pathogenes begins with adhesion of the conidia to the cuticle, germination and penetration, followed by development of the fungus inside the host, leading to death of the host under suitable condition .Death is followed by external sporulation which helps to spread the fungus and establish an epizootic which may result in very high level of Kill.

Among the biotic factors the population density may also be an important factor for pathogenicity of BPH. The correlation studies in Bankura, Birbhum and Nadia showed that the BPH population had a significantly negative correlation with the percent infection by fungi (Table 3). It is evident from Table 4 that there is distinct variation in the mortality of BPH due to application of mix culture consortium of different fungal CFU in the laboratory. The significantly highest mortality of BPH (V instar nymph) could be observed at 1 x 10^6 per ml (20.64%). The next in order was 0.5 x 10^6 per ml (14.758) followed by 0.25 x 10^6 per ml (6.770) and were significantly lower than 1 x 10^6 per ml. With no use of fungal spore the per cent mortality of BPH was 4.05 which was significantly lower than the others treatments. The development of insect fungi as mycoinsecticide has received serious attention as means for insect control [22,23].

Table 3: Values of correlation coefficient (r) of pathogenicity (%) of some natural fungus with the prevailing temperatures humidity a population of BPH

Fungi	**Red and laterite Zone(Bankura)**					
	Tempeature		**Relative humidity**		**Rainfall**	**Biotic factor**
	Max	**Min**	**Max**	**Min**		**Population of BPH**
***Aspergillus flavus* (Link)**	**0.703***	**0.735***	**0.833***	**-0.925***	**-0.932***	**-0.997**
	Red and laterite Zone(Birbhum)					
***Aspergillus flavus* (Link)**	**0.262**	**0.408**	**0.459**	**-0.504**	**-0.728***	**-0.946***
	New alluvial zones(Burdwan and Nadia)					
***Aspergillus flavus* (Link)**	**0.221**	**0.868***	***0.887***	**-0.891***	**-0.892**	**-0.868**

* Significant P < 0.05, ** Significant P < 0.01

The mortality after spraying of crushed fungal spore over fresh BPH colony in laboratory are given in table-4

Table 4: Efficacy of natural fungus against BPH in laboratory

Mean for spore formulation			
1 x 10^6 per ml	**0.5 x 10^6 per ml**	**0.25 x 10^6 per ml**	**0 x 10^6 per ml**
20.641	14.758	6.770	4.050

Mean for base		
Talc based	**Water suspension**	**Water dispersible powder**
16.004	16.004	10.609

4. Statistical analysis

Source of variation	**C. D. at 0.05%**
Spore (A)	0.210
Base (B)	0.565
A x B	0.863

There is a clear variation in mortality at 5% statistical level of significant due to use of different base with mix culture consortium in the present investigation. The % mortality was highest (16%) in Talk based followed by wettable powder of china clay (10.60%) and were significantly higher than water suspension (8.05%). The interaction between two factors i.e. spore (A) x base (B) was also statistically significant.

5. Conclusion

From the result it is to be concluded that the importance of common soil saprophytes as entomopathogen under natural condition could not be over ruled from the evidences obtained in the present investigation. Application of single fungal species as bio-control agents may however fail to be effective due to their specific growth requirements like temperature, relative humidity etc. This mixed culture consortium may be a successful bio-control agent against BPH field conditions. The fungi observed to colonize the infected BPH under field condition were amorphic (asexually reproducing) in nature. Moreover under field condition at least 5 fungal species were observed to colonise the dead BPH population. Even a single dead insect was found to be colonized by more than one fungus also. However the exact fungal species responsible for the mortality was not established during the present investigation. The fungal species isolated from the dead BPH were mostly soil saprophytes but they have the capacity to colonise insects.

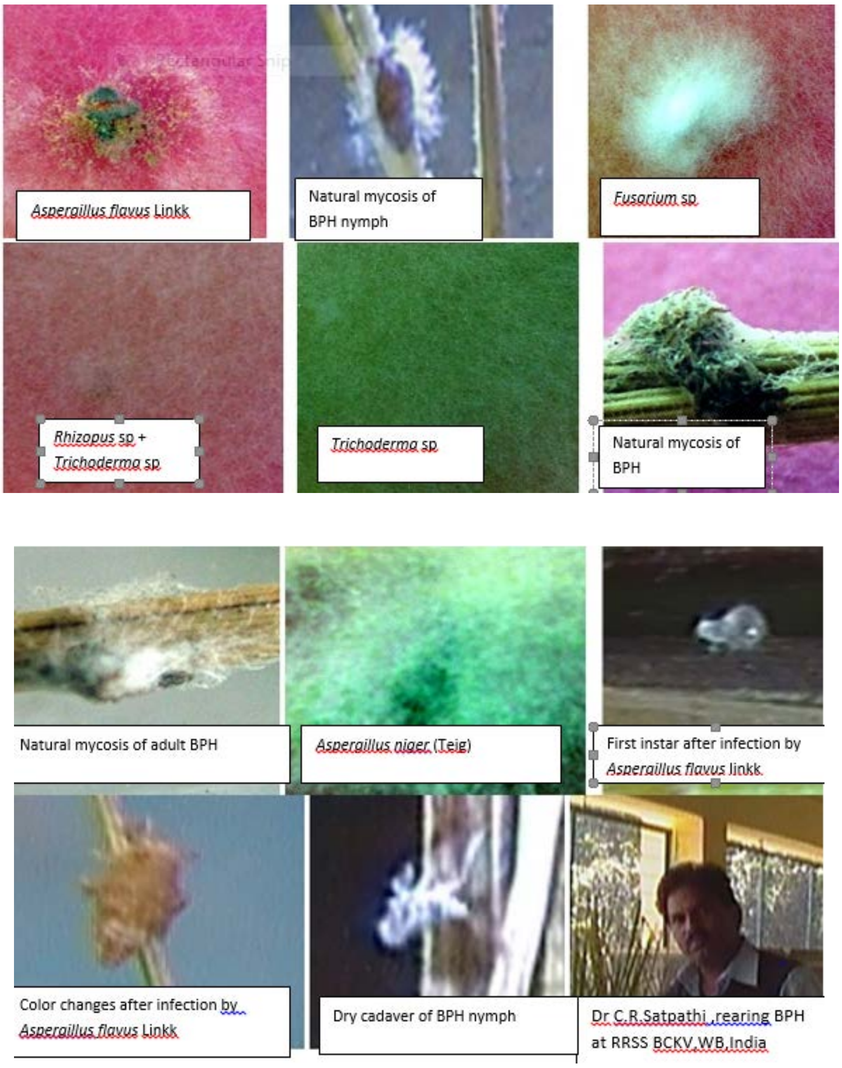


Figure 2: Photographs of different fungal pathogens isolated from BPH

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