Eco. Env. & Cons. 28 (1) : 2022; pp. (416-421)
Copyright@ EM International
ISSN 0971–765X
DOI No.: http://doi.org/10.53550/EEC.2022.v28i01.063

A Report on Isolation of mycotoxigenic Fungus from the Larval Body Surface of Paddy Pest, Yellow Stem Borer, *Scirpophaga incertulas* (Walker) (Pyralidae : Lepidoptera)

Eureka Mondal[1]*, Gautam Chakraborty[2] and Kaushik Chakraborty[1]

[1]Department of Zoology, Raiganj University, Raiganj, Uttar Dinajpur, West Bengal, India
[2]Department of Agricultural Entomology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

(Received 21 May, 2021; Accepted 30 June, 2021)

ABSTRACT

Yellow stem borer,*Scirpophaga incertulas* Walker (Lepidoptera: Pyralidae) is considered as major paddy pest of global importance.For investigating the role of *S.incertulas* as mechanical vector of fungal pathogen, experiments in field condition were carried out at two different places, *viz.* Central Research farm, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Nadia, West Bengal and Tarakeswar, Hooghly, West Bengal during two consecutive *kharif* seasons of 2018-2019.The infestation by *S.incertulas* was identified in terms of the incidence of Dead Heart (DH) produced during vegetative growth stage of paddy crop. Twenty DH sample were collected and brought to the Laboratory of Dept. of Zoology, Rabindra Mahavidyalaya, Champadanga, Hooghly, West Bengal. Then the DH stem portions were splitted meridionally, larvae of different age groups were collected and stored them within a test tube. Few drops of the suspension made from the washing of the body surface of the larvae were inoculated randomly in Petri plates containing Potato dextrose agar (PDA) media that was incubated at 26 ± 2°C for 6-7 days. One fungal isolate was obtained in pure culture. Morpho-taxonomy reveals the isolate was *Aspergillus flavus*. It is thus evicted that apart from paddy crop damage, *S.incertulas* also acts as a carrier of potent mycotoxin producing fungal pathogen *Aspergillus flavus*.

Key words *: Paddy, Fungus, Dead Heart, Mycotoxins.*

Introduction

Rice, *Oryza sativa* L.(Family: Poaceae) is regarded as the most important food crop providing half food security for the total global population (FAO, 2011) and Garris *et al*. (2005). About 2.5billion people of Asia solely depends on rice as their principal food staff and about 90% of the rice is cultivated in Asia (Khush *et al*., 2002). 'Rice' consists of one fifth of the total world crop land that are used for cereal production (Pathak, 1994).

According to Matteson (2000), in the tropical Asian countries, low yielding of rice results from the damages by the insect pest population. Nearly 132 species of insect pests are recorded to ravage the rice fields. Out of these, about 15 to 20 insect species are very much economically important (Kalode, 2005). Among them, stem borers (SBs) are principal group of insect pests of rice (Dhaliwal *et al*., 1996). Yellow stem borer (YSB), *Scirpophaga incertulas* Walker (Pyralidae:Lepidoptera) is considered as

***Corresponding author's email:** eurekazoology10@gmail.com

the most notorious and dominating species out of the all species of SBs throughout India (Mahar *et al*.,1985). It has been reported that YSB infestation covers all the agro-ecological regions of West Bengal and comprising about 89.50% of the total SB population (Biswas, 2006). According to Sarwar (2012) YSBs are monophagous. Kakde *et al.*(2014) has reported that during the reproductive growth period of crop, larval infestation in rice causes dry ing of the central shoot known as 'dead heart' (DH) in young plants. Massive damage of the growing panicle results in 'white ear head' (WH). In case of severe infestation, YSB results in 100% crop destruction (Kushwaha, 1995).

After entering within the paddy stem by successful boring/tunnelling, the larvae matures and subsequently pupates (Sarwar, 2012). The endophyte may undergo physiological alterations, from a mutualistic to a pathogenic interaction or vice versa, depending on several factors such as drought, excessive humidity, stress and poor nutrient supply (Millar, 1980 and Fisher *et al*., 1992).

Endophytic fungi colonise healthy plant tissues but do not always cause noticeable symptoms. Thisinteraction is regarded as mutualistic (Carroll, 1988).Schulz and Boyle (2005), however, had noted that mutualistic interaction may only be temporary and is subject to change over time. Therefore, endophytic fungi could account for those fungi with an epiphytic phase as well as latent pathogens that live asymptomatically in the host plant for some time in their life (Leslie, 1990).

Studies have done on endophytic fungi in different agricultural crops, such as maize (Fisher *et al.,* 1992), banana (Cao *et al*., 2002), coffee (Santamaria *et al.,* 2005) and wheat (Larran *et al.* 2007) but observation on paddy plant is scanty. *Fusarium*, *Aspergillus* and *Penicillium* were the most common endophytic fungal genera recovered from healthy paddy plant in China (Tian *et al*., 2004). *Penicillium chrysogenum* and *F.oxysporum* were re- ported from healthy leaves and roots of paddyplants, (Shankar *et al*., 2009). Rodolfi *et al*. (2006) re- ported that endophytic colonisation of seeds by *Penicillium, Fusarium* and *Aspergillus*on more than one Italian rice cultivar. For most endophytic fungi, the types of interactions between the microbe and the host are described as an interaction at a particular point of time or momentary status (Schulz *et al.,* 2005). An endophytic fungus could act as a latent pathogen in the plant tissues until changes in environmental fac-tors or decline in host defence mechanisms allow the endophyte to become pathogenic (Bayman *et al.,* 2007). Thus, the assemblage of endophytic fungi from paddy plants may indicate that some of the fungi such as *Fusarium*, *Aspergillus, Curvularia* and *Penicillium* are possible latent pathogens (Fisher 1992). Species of *Fusarium* have been isolated from paddy grains (Abdel-Hafez *et al.,* 1987), paddy and milled rice (Tonon *et al*., 1997) and paddy seeds (Pacin *et al.,* 2002). Plant pathogenic species of *Fusarium* have been known to be associated with several types of paddy disease such as bakanae, seedling rot and root rot. According to Fisher *et al. (*1992), two species of *Fusarium*, *F. equiseti* and*F.oxysporum* have been obtained from healthy paddy plants.

Studies focused on endophytic fungi colonising healthy paddy tissues but probably no convincing report has been obtained from insect infested partsof paddy crops. Thus, the objective of the present study is to assess the endophytic fungal assemblages in unhealthy, *i,e.*YSB pest infested paddy plants.

Studies have focused on endophytic fungal assemblage on healthy paddy tissues but report on role of YSB as mechanical vector is very scanty. Thus, the present investigation aims to elaborate the role of YSB as a carrier of pathogenic fungus like *Aspergillus* on the insect body surface.

Materials and Method

Field layout and Experimental Setup

Field experiment was conducted with transplanted 30-days old healthy seedlings of widely cultivated variety Lalat (*IET-9947*) and were laid in a completely randomised block design with three replication during two consecutive *kharif* seasons of 2018-2019 at two different places *viz.* Central Research farm, BCKV, Nadia, WB (22.9452° N, 88.5336° E) and Tarakeswar, Hooghly, WB (22.8891° N, 88.0180° E).

Assessment on pest infestation

The infestation by YSB was identified in terms of the incidence of DH produced during vegetative stage.

(a) (b)

Fig. 1. (a) Selection of Dead Heart (DH) in the paddy field (b) Isolation of yellow stem borer larvae from DH portion

Twenty of them were randomly collected and brought to the laboratory of Dept. of Zoology, Rabindra Mahavidyalaya, Champadanga, Hooghly, WB (22.8379° N, 87.9739° E). Then the DH stem portion were splitted meridionally, larvae of different age groups were collected from the portion and stored them within a test tube (Fig.1).

Isolation of Fungus

The larvae, stocked within the test tube were then washed in sterile distilled water properly for 1 min. Few drops of the suspension from the tube (after proper shaking) was taken and inoculated randomly in Petri plate, containing Potato dextrose agar (PDA) media. An antibacterial agent Chloramphenicol (50 ppm) was used to inhibit the growth of unintended bacteria.

Petri plate was incubated at 26 ± 2 °C for 6-7 days and examined daily for fungal growth. Mycelia growing out from the plant tissues were then subcultured on PDA to obtain pure culture and used for identification. Morphological and cultural characteristics of the growing cultures were evaluated for preliminary identification (Fig. 2).

Morphological characterization

Fungal morphological studies consisted of mycelium growth, colour, cellular contents and characters of fruiting bodies of fungi. Macroscopic characters like fungal colony growth, size, colour, texture, shape, reverse colour, exudates, and margin of the colony were noted for fungal (Barnett, 1998). Microscopic characterization of the fungal isolates was done by making the slides of different fungal species (Fig.3).

Confirmation of the identification was done by National Fungal Collection and Culture of India (NFCCI), Agharkar Research Institute, Pune, India. Mycelia sterilia fungi were identified using internal transcribed spacer region of ribosomal DNA (ITS1-5.8S-ITS2). Polymerase chain reaction (PCR) and sequencing procedures, as well as the primer sequences used, were as previously described by White *et al*. (1990).

Results and Discussion

Morphological characterization of fungus

Following the subculture, single species of fungus was primarily identified. Apparently it was green in colour with yellow shades and the texture was usually loose and velvety. The colony turns brown in colour after passing a few days (Fig.2).

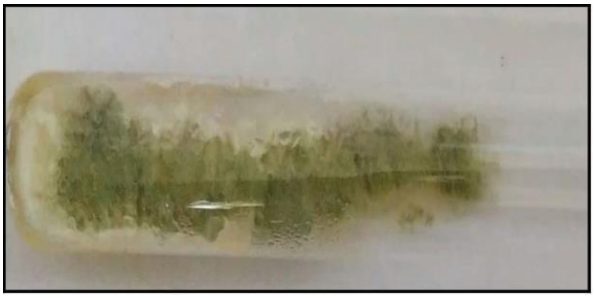

Fig. 2. Macroscopic view of the Fungal culture

Observation on microscopic features of *A. flavus* revealed that the colonies were biseriate with philiades radiating in all sides from metulae and attached subglobose or globose vesicles of variable size. Somewhat similar characteristics were reported by Tathana *et al.,* (2017). Conidial spores were also produced on the conidiophore vesicles. The conidial spores have a thick mycelial mat having a size of 3 to 6 µm. The

conidiophore originates from the hyphae that occur as thread-like septate branches that form mycelium (Fig.3). The conidia had a globose shape ranging between 250 μm and 450 μm in diameter with thin walls and rough texture. Odhiambo *et al.,* (2013) stated that conidiophores of *Aspergillus flavus* had a rough texture and were unbranched.

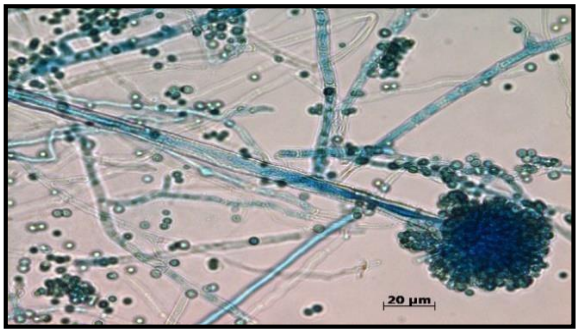

Fig. 3. Microscopic view of the Fungal culture

Molecular characterization of fungus (As per Altschul *et al.,* 1990)

Fungal strain in the present study showed 99% sequence similarity with ***Aspergillus flavus.*** Sequence analyses with NCBI accession number LC385527.1, ***Aspergillus flavus*** AGERI-S4 resulted in following alignment statistics. Alignment statistics: Query Length - 538, Score - 971 bits (1076), Expect - 0.0, Identities – 538/538(100%), Gaps - 0/538 (0%), Strand -Plus/Minus (Table1.). The sequence has obtained and the identity of isolates were confirmed by BLAST analysis (Table 2.). The BLAST analysis revealed that the identity of isolated fungus as *Aspergillus flavus,* and showed the 99% similarity with other extype isolates sequences present in NCBI data base. The isolate Sample is closely related to the NCBI isolate (LC385527.1).

Table 1. Multiple sequence alignment

```
Query  1    GGAGGTTTTACGGCAGGCTGCAGGGGCCACTACAGAAGCGAGAAGAAAACTACTACGCTG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  486  GGAGGTTTTACGGCAGGCTGCAGGGGCCACTACAGAAGCGAGAAGAAAACTACTACGCTG  427

Query  61   AGAGTGTGCTCCAGCACCGCCACTAACTTTGAGGAGATACGCTGTAGACGTAGGCTCCCA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  426  AGAGTGTGCTCCAGCACCGCCACTAACTTTGAGGAGATACGCTGTAGACGTAGGCTCCCA  367

Query  121  ACGCTAAGCGACAGAGGCTTAAGGGTTGAAATGACGCTCGAATAGGCATGCCCACTAGAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  366  ACGCTAAGCGACAGAGGCTTAAGGGTTGAAATGACGCTCGAATAGGCATGCCCACTAGAA  307

Query  181  TACTAATGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  306  TACTAATGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACA  247

Query  241  TTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  246  TTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGA  187

Query  301  AAGTTTTGACTTATTTTATTTAAGCAATCAGACATACTTGTGTACATACAAGAGTTTAGC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  186  AAGTTTTGACTTATTTTATTTAAGCAATCAGACATACTTGTGTACATACAAGAGTTTAGC  127

Query  361  TGTCCACCGGCGGGCCATGTGCGTGCTACGCGGCAGCTACAGGGGAGCCACAGGGTAGCC  420
            |||||||||||||||||||||||||||||||||||||||||||| ||| ||||||||||||
Sbjct  126  TGTCCACCGGCGGGCCATGTGCGTGCTACGCGGCAGCTACAGGGTAGCTACAGGGTAGCC  67

Query  421  GCGGCTCGCGCCG  433
            |||||||||||||
Sbjct  66   GCGGCTCGCGCCG  54
```

Table 2. Top five hits upon BLASTn analysis

Gene Bank Accession No.	Description	Max score	Query cover	Query coverage	E value	Identity (%)
LC385527.1	*Aspergillus flavus* AGERI-S4	971	971	100%	0.0	100%
MF346058.1	*Aspergillus sp.* isolate FG41	971	971	100%	0.0	100%
MF346056.1	*Aspergillus sp.* isolate B261	971	971	100%	0.0	100%
MH279454.1	*Aspergillus flavus* isolate DTO 389-C1	971	971	100%	0.0	100%
MH279453.1	*Aspergillus oryzae* isolate DTO 389-C2	971	971	100%	0.0	100%

Conclusion

From the study, it has been concluded that, larval instars of *Scirpophaga incertulas* not only damages the growing paddy crop, but also act as an important carrier of potentially pathogenic fungus like *Aspergillus flavus. A.flavus* is a mycotoxin producing harmful fungus. Such aflatoxin producing pathogenic fungus is responsible for remarkable biochemical changes that may affect the growth and development of other insect pests in field condition.

However to apply *A.flavus* as entomopathogenic and biocontrolling agent at mass scale to check insect pest further investigation is required.

Acknowledgement

The authors full heartedly acknowledge the support of the faculty members of the concerned departments from whom guidance and suggestions were taken as and when needed to carry out the experiment. Acknowledgement is also forwarded to National Fungal Collection and Culture of India (NFCCI), Agharkar Research Institute, Pune, India for the identification of fungal culture.

References

Abdel-Hafez, S., El-Kady, I.A., Mazen, M.B. and El-Maghraby O.M.O. 1987. Mycoflora and trichothecene toxins of paddy grains from Egypt. *Mycopathologia*. 100 (2) : 103–112.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman D.J. 1990. Basic local alignment search tool. *Journal of Molecular Biology*. 21 (5) : 403-410.

Barnett, H. L., Barry, B. and Hunter, F.1998. *Illustrated Genra of Imperfect Fungi* (4th Ed.). American Pathology Society. USA.

Bayman, P., Kubicek, C.P. and Druzhinina, I.S. 2007. *The Mycota IV: Environmental and Microbial Relationships*. 2nd ed. New York: Springer-Verlag.

Biswas, S. 2006. Studies on the stem borer, leaf folder and gall midge under terai agro-ecology of West Bengal. M.Sc (Agriculture) Thesis submitted to the Uttar Banga Krishi Viswavidyalaya. 1-46.

Cao, L.X., You, J.L. and Zhou, S.N. 2002. Endophytic fungi from *Musa acuminata* leaves and roots in South China. *World Journal of Microbiology and Biotechnology.* 18(2) : 169–171.

Carroll, G.C. 1988. Fungal endophytes in stems and leaves From latent pathogens to mutualistic symbiont. *Ecology*.69:2–9.

Dhaliwal, G.S. and Arora, R. 1996. An estimate of yield losses due to insect pests in Indian Agriculture. *Indian Journal of Ecology*. 23 : 70-73.

FAO. 2011, Food and agricultural commodities production. Food and Agriculture Organization of the United Nations.

Fisher, P.J. and Petrini, O. 1992. Fungal saprobes and pathogens as endophytes of rice (*Oryza sativa* L.) *New Phytologist.* 120 : 137–143.

Garris, A.J., Tai, T.H., Coburn, J., Kresovich, S. and Mccouch, S. 2005. Genetic structure and diversity in *Oryza sativa* L. *Genetics.* 169 :1631-1638

Kakde, A.M. and Patel, K.G. 2014. Seasonal Incidence of Rice Yellow Stem Borer (*Scirpophaga incertulas* Wlk.) In Relation to Conventional and Sri Methods of Planting and Its Correlation with Weather Parameters, IOSR. *Journal of Agriculture and Veterinary Science.* 7 : 05-10.

Kalode, M.B. 2005. Insect pest of rice and their management in Rice in Indian Perspective edited by SD. Sharma and BC. Nayak. Today and Tomorrow Printers and Publishers (India). 819-854.

Khush, G.S. and Brar, D.S. 2002. Biotechnology for rice breeding. In proceeding of the 20th session of the International Rice Commission.

Kushwaha, K. S. 1995. Chemical control of rice stem borer,*Scirpophaga incertulas* and leaf folder, *Cnaphalocrocis medinalis* on Basmati. *Journal of Insect Science.* 8(2): 225-226.

Larran, S., Perello, A., Simon, M.R. and Moreno, V. 2007. The endophytic fungi from wheat (*Triticium aestivum* L) *World Journal of Microbiology and Biotechnology*. 23 (4) : 565–572.

Leslie, J.F., Pearson, C.A.S., Nelson, P.E. and Tousson, T.A. 1990. *Fusarium* species from corn, sorghum and soybean fields in the central and eastern United States. *Phytopathology*. 80 : 343–350.

Mahar, M.M., Bhatti, I.M. and Dhuyo, A.R. 1985. Stem borer infestation and yield loss relationship in rice and cost- benefits of control. Paper presented at 5th National Seminar on Rice and Production. Kalashakaku. 23-25.

Matteson, P.C. 2000. Insect pest management in Tropical Asian Irrigated rice. *Annual Review of Entomology.* 45: 549-574.

Millar, C.S. 1980. Infection processes on conifer needles. pp. 185–209. In: Blakeman JP, editor. Microbial ecology of the phylloplane. London: Academic Press.

Odhiambo,B.O., Murage,H. and Wagara,I.N., 2013. "Isolation and characterisation of aflatoxigenic *Aspergillus* species from maize and soil samples from selected counties of Kenya," *African Journal of Microbiology Research*. 7(34):4379–4388.

Pacin, A.M., Gonzalez, H.H.L., Etcheverry, M., Resnik, S.L., Vivas, L. and Espin, S. 2002. Fungi associated with food and feed commodities from Ecuador. *Mycopathologia.* 156 : 87–92.

Pathak, M.D. and Khan, Z.R. 1994. Stem borers In: Insect pest of rice. IRRI & ICIPE. 5-17.

Petrini, O., Fisher, P.J. and Lappin Scott, H.M. 1992. The distribution of some fungal and bacterial endophytes in maize (*Zea mays* L.) *New Phytologist*. 122 :299–305.

Rodolfi, M., Lorenzi, E. and Picco, A.M. 2006. Fungal pathogens on Italian rice (*Oryza sativa* L) seed. 3rd International Seed Health Conference of Seed Pathology Section; Bydgoszcz, Poland. 6–8 pp. 76–77.

Rodrigues, A.A.C. and Menezes, M. 2005. Identification and pathogenic characterization of endophytic *Fusarium* species from cowpea seeds. *Mycopathlogia.* 159:79–85.

Santamaria, J. and Bayman, P. 2005. Fungal epiphytes and endophytes of coffee leaves (*Coffee arabica*) *Microbial Ecology*. 50(1) : 1–8.

Sarwar, M. 2012. Management of rice stem borers (Lepidoptera: pyraridae) through host plant resistance in early, medium and late planting of rice (*Oryza sativa*. L). *Journal of Cereals and Oil Seeds.* 3(1) : 10-14.

Schulz, B. and Boyle, C. 2005. Fungal endophyte continuum. *Mycological Research.* 109 : 661–686.

Shankar, N.B., Shashikala, J. and Krishnamurthy, Y.L. 2009. Study on the diversity of endophytic communities from rice (*Oryza sativa* L.) and their antagonistic activities in vitro. *Microbiological Research.* 164(3): 290–296.

Thathana, M., Murage, H., Abia, A. and Pillay, M. 2017. "Morphological characterization and determination of aflatoxinproduction potentials of *Aspergillus flavus* isolated from maize and soil in Kenya," *Agriculture.* 7(10):80.

Tian, X.L., Cao, L.X., Tan, H.M., Zaeng, Q.G., Jia, Y.Y., Han, W.Q. and Zhao, S.N. 2004. Study on the communities of endophytic fungi and endophytic actinomycetes from rice and their antipathogenic activities in vitro. *World Journal of Microbiology and Biotechnology*. 20(3): 303–309.

Tonon, S.A., Marucci, R.S., Jerke, G. and Garcia, A. 1997. Mycoflora of paddy and milled rice produced in the region of northeastern Argentina and sourthern Paraguay. *International Journal of Food Microbiology*. 37(2) : 231–235.

White, T.J., Bruns, T., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Inc; pp. 315–322. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: A guide to methods and applications. San Diego: Academic Press.