Longevity of *Bipolaris oryzae* on HEERA-2 Hybrid Rice Seed

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Abstract
Seeds of hybrid rice variety Heera-2 was collected from Mymensingh district in Bangladesh. Longevity of *Bipolaris oryzae* was studied in 2012 to 2016 at Seed Pathology Centre, Bangladesh Agricultural University, Seed processing &preservation center and Seed Pathology Laboratory of Supreme Seed Company Limited. The data collected from the experiments were analyzed for test of significance and compared the treatment means by using DMRT at 5% level of probability following the Statistical tool for Agricultural Research (STAR) 2.1 program.The longevity of *B. oryzae* was observed till five years by blotter incubation methods, seedling symptom test and growing on test. Both untreated and seeds treated with Vitaflo 200FF (carboxin and thiram) were kept in jute bag and polybag stored for five years under both normal and dehumidified condition. The inocula of *B. oryzae* on Hybrid Heera 2 seed remained viable up to five years. When the seed samples were stored under dehumidified condition, the highest incidence of *B. oryzae* was recorded in case of jute bag after five years. After treated with Vitaflo 200FF, there was no any infection of *B. oryzae* under both storage condition

Key words: *Bipolaris oryzae,* Heera-2, Hybrid rice, Longevity, Seed

Introduction

Rice is one of world's most promising staple food grains and about more than 90% of the world's rice is produced and consumed in Asian countries (Singh *et al.* 2013). The yield of high yielding rice varieties is comparatively low. Hybrid rice varieties have high yield potential. So, emphasis should be given for cultivation of hybrid varieties (Awal *et al*. 2007). In the wake of a big flood in 1998, Government of Bangladesh decided to introduce hybrid rice cultivation and strong participation from private sector. Now in 2020, of the total 201 hybrid rice varieties released by the Government, 170, 25 and 6 belong to Boro, Aman and Aus season, respectively. Out of 201 varieties 186 supplied by private seed companies & NGOs and 15 supplied by Public sectors, respectively. Supreme Seed Company ltd is the leading private Company for supply of maximum quantity of hybrid rice seeds to the farmers. Quality seed is the crying need of the day. As rice contributes 77% of the total annual crop production in our country, seed quality in rice plays an important role to achieve self-sufficiency and food security. In Bangladesh, supply of quality seed is around 20% of the total national seed requirement. In case of rice 25-30% of seed of the total produced can be considered as quality seed (Huda, 2001) and this quality is judged only physical purity, germination and seed moisture content. Health is not considered as a parameter of seed quality. But infected unhealthy seeds, harboring seed-borne pathogens, fail to germinate or the young seedlings emerging from the infected seeds die after germination resulting post-emergence death or damping off and seedling blight. The fungus *Bipolaris oryzae* causes different diseases in rice (*Oryza sativa* L.) such as leaf brown spot, grain brown spot and seedling death (Ou, 1985; Lee, 1992). The damage caused by this pathogen with regard to yield is associated with the disease incidence on the leaves and grains (Lee, 1992). The occurrence of brown spot on leaves affect grain production by decreasing the number and the grains weigh per panicle (Aluko, 1975).The range of reported yield losses to brown spot, often expressed in relative terms, is variable from 4 to 29% (Bedi and Gill, 1960), about 12% (Aluko, 1975) from 8 to 23% (Fomba and Singh, 1990) from 26 to 52% (Chakrabarti, 2001). The latter figures represent a broader and higher range because it accounts for losses caused by grain infection. Heavily infected grains are not suitable for human consumption, which may party explain the impact of brown spot in the Great Bengal Famine. Seed deterioration during storage is a gradual and inevitable process causing considerable losses. Seeds tend to lose viability and vigor during storage and information on storability of seed lots from harvest until the next season and also for carry over purposes is of immense importance in any seed production program. Availability of good quality seed of parental lines is essential for any successful hybrid seed production. Seed micro-flora has been recognized as an important factor responsible for deterioration in quality of seeds during storage (Gupta and Aneja, 2001). Hence, an understanding of how best these seeds can be stored at a relatively low cost with minimal deterioration in quality for periods extending over one, two or more seasons will be of great interest to the seed industry. Seed longevity depends upon a number of factors such as the genetic constitution, initial seed quality, storage

environment, packaging material and pre-storage seed treatments. So, objectives of this research to study the longevity of *B. oryzae* in hybrid rice seeds by pre storage seed treatment and interaction thereof with storage condition and containers.

Materials and Methods

Longevity of seed-borne fungi was studied at the Seed Processing and Preservation Centre Trishal, Mymensingh and Seed Testing Laboratory, Uttara, Dhaka of Supreme Seed Company Ltd. during June 2012 to June 2017. A seed lot having higher percentage of infection of *B. oryzae* was selected. The lot of Heera-2, a cultivar highly prone to seed born *B. oryzae* was used. Seed treating fungicide Vitaflo (Carboxin 17.5% & Thiram 17.5%) was seed treatment against *B. oryzae.* Both treated (with vitaflo) and untreated seeds of Heera-2 were used. Twenty kg of seeds was taken at random. The sample was divided in to four parts and taken in two in Jute bags & polybags separately. One half of both Jute bag & poly bag were stored in the laboratory under normal condition where temperature ranges was 11°C to 40^0C, and relative humidity 40% to 98%. The other half was stored in the dehumidified chamber at temperature 18±2°C and 40% RH for five years.

Table 1. Seed stored under different storage conditions

Treatment	Storage Condition	Storage Container
Untreated	Normal (Tem11°C – 40^0C) (RH 40%-98%)	Jute bag
		Poly bag
	Dehumidified (Tem 18±2°C) (RH 40%)	Jute bag
		Poly bag
Treated With Vitaflo (carboxin + thiram)	Normal (Tem11°C – 40^0C) (RH 40%-98%)	Jute bag
		Poly bag
	Dehumidified (Tem 18±2°C) (RH 40%)	Jute bag
		Poly bag

Blotter incubation methods

Four hundred seeds from each of the four seed sample were tested to examine the viability of *B. oryzae* on the infected seed. Blotter incubation method at four months' interval was used. Number of incubated seeds with the growth of *B. oryzae* was observed under sterio microscope at 25 X and counted according to the keys outlined by Mathur and Kongsdal (2003). The percentage of seeds with the growth of *B. oryzae* on each sample in each test was compared and duration of viability was determined. The experiment was continued for five years.

Seedling symptom test

Longevity of *Bipolaris oryzae* was studied under control environment in growth chamber using test tube agar methods. The infected seed was obtained from the seed lots collected from the same sample. Symptoms on the seedling was recorded and the pathogen was isolated and identified following the keys outlined by Mathur and Kongsdal (2003). Percentage of the seed/seedling showing the growth/sporulation of the pathogen was calculate.

Growing on test

Bipolaris oryzae infected seed lots were identified based on the result of previous experiments. Four hundred seeds were taken from the working sample for testing its germination capacity. The infected seedlings were surface-sterilized with 1% sodium hypochlorite (NaOCl) and sown the sterile sand in pastic box using 100 seedlings/sample. Air was blown in the polyethene bag by mouth before sealing the bag with a rubber band so that seedling do not touch the plastic. The disease was also monitored after 30 days by disease washing the seedlings to remove sand from the box. The number of infected seedlings were counted and confirmed by isolation of *Bipolaris oryzae.*

Results

Blotter incubation methods

Longevity of *Bipolaris oryzae* was recorded when the Heera-2 seed samples were stored under dehumidified condition, the highest result was recorded in jute bag 18% of *B. oryzae* which was significantly different from others)and 15% in poly bag after 60^{th} months of storage. Incidence of *B. oryzae* significantly decreased after 12^{th} months of storage in normal condition. Seed stored in polybag under normal condition result was decreased after 6^{th} month of storage and the lowest 3% incidence was found after five years. After 24^{th} months of storage in all conditions non-significant results was found. The decreased in incidence of *B. oryzae* was observed during storage in Vitaflo treated seeds. After treated with Vitaflo fungicides data recorded that there was no infection of *Bipolaris oryzae,* in both storage conditions viz. normal and dehumidified and in both containers viz. jute bag and poly bag till five years with six months' interval. After five years of storage highest germination of *B. oryzae* infected hybrid rice was recorded when seed treated with Vitaflo 200 FF and kept in polybag under dehumidified condition (Monira *et al.* 2020). Biradarpatil and Shekhhargauda (2007) studied that seed dressing fungicide like thiram protecting the seeds from hazards of storage fungi. The result supported that Suzuki *et al.* (1930) recorded longevity of *B. oryzae* within rice seed is 10 years and it can survive within the seed for four years (Kumari *et al.* 2015). Untreated seed kept in both jute and polybag under normal condition incidence

of *B. oryzae* was significantly decreases after 12th months of storage.

(a)

(b)

Fig. 1. (a) & (b). *Bipolaris oryzae* after five years on heera 2 hybrid rice seeds

Table 2. Longevity of *Bipolaris oryzae* on Heera-2 hybrid rice seed at storage

Storage Condition	Storage Container	Incidence % of *B. oryzae* at different duration/months											CV (%)
		1st	6th	12th	18th	24th	30th	36th	42th	48th	54th	60th	
Dehumidified (Tem 18±2^{0}C) (RH 40%)	Jute bag	25 A	25 aA	25 aA	24 A	24 A	22 aAB	22 aAB	20 aAB	20 aAB	20 aAB	18 aB	8.23
	Poly bag	25 A	24 aA	22 bAB	23 bA	22 bAB	20 aABC	19 aABC	16 bBC	16 bBC	16 bBC	15 bC	10.86
Normal (Tem 11^0-40^{0}c) (RH 40%-98%)	Jute bag	25 A	24 aA	22 bAB	20 BC	18 C	12 cD	12 bD	10 cDE	10 cDE	10 cDE	8 c	7.76
	Poly bag	25 A	22 aAB	20 bABC	20 ABC	18 BCD	15 bCDE	13 bDEF	10 cEF	8 cFG	8 cFG	3 dG	12.28
	CV (%)	8.72	7.29	5.95	8.6	12.91	9.17	10.9	11.3	11.1	12.8	11.1	

Seedling symptom test

Longevity of *B. oryzae* was observed by using seedling symptom test Heera-2 seedlings. Incase of all varieties incidence% of *B. oryzae* was observed after five years (figure 2). In Heera-2, 25% after five years, whereas, 72% was at initial stage. The data was recorded 22 %after five years on Heera-2 rice seeds. Data has been recorded that longevity of *B. oryzae* was found on heera 2 rice varieties till five years using by blotter incubation methods, Longevity of *B. oryzae* by seedling symptom agar test tube methods symptom on the coleoptile and roots were observed. There was a significantly different on the occurrence of symptom on coleoptiles and root of seedling in case of all varieties. Highest symptoms were observed on coleoptiles 42%.Thomas,1940 reported that the coleoptile and sometimes roots are often infected from diseases seeds but lesions may not be produces a subsequently developed leaves on account of rapid growth of the leaves under normal conditions. Infection may also take place from the soil. Kuribayshi (1929) also studied that the fungus can survive in the infected grains for varying length of time depending on the storage of 2 years. The longevity of *B. oryzae* and the survival of host seed are intimately related. The conditions under which seeds stored may affect the longevity of the pathogen in/on the seed. Besides, the intrinsic nature of the organism and its location on or in the tissues of seeds also affect to the longevity of organism. (Maude, 1996). Ou (1989) writes that primary infection through diseased seed is probably most common, diseased seeds do not always give rise infected seedlings.

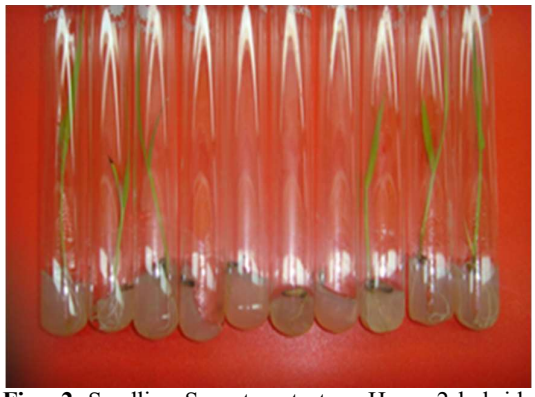

Fig. 2. Seedling Symptom test on Heera 2 hybrid rice seed

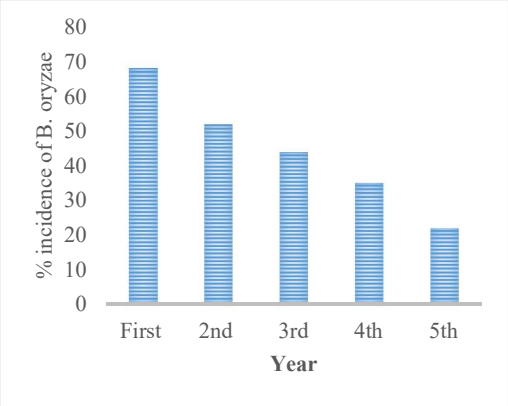

Fig. 3. Longevity of *B. oryzae* using by Seedling symptom test on heera 2 hybrid seed

Growing on test

Bipolaris oryzae was found on Heera 2 till five years by growing on test. Incidence % was recorded 6, after 5th years, when initial incidence% of *B. oryzae* was recorded 22%, Heera-2 rice seed. *Bipolaris oryzae* was found till five years by growing on test. Incidence % was recorded 6%, when initial incidence% of *B. oryzae* was recorded 22% on Heera-2 hybrid rice seed. Infection of *Bipolaris oryzae* was recorded by using growing on test from Hybrid rice. Gupta and Aneja (2004) recorded that during storage, especially under an ambient environment, seeds produce change due to fungal activity, resulting in deterioration of their quality. According to Rennie (1998) the successful transmission of pathogen to seedling depended not only on the amount and location of inoculum in/ on the kernel but also environmental conditions during germination and seedling establishment. However, the similar results were also obtained by Imolehin (1983), Kulik (1977) and Malavolta *et al*. (2002) showed significant correlation between incidence of *B. oryzae* and seed germination and seed incidence and death or infected seedling.

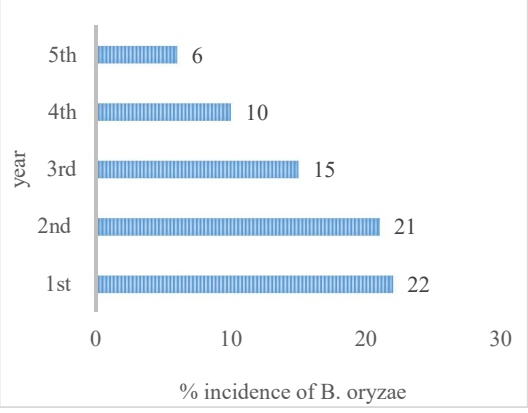

Fig. 4. Longevity of *B. oryzae* using by growing on test on Heera-2 hybrid seeds

Conclusions

The inocula of *B. oryzae* on Hybrid Heera 2 seed remained viable up to five years. After five years, the incidence of *B. oryzae* was 15-28% in four varieties stored in dehumidified chamber in jute bag while it was 8-20% stored under normal condition in polybag. After treated with vitaflo fungicides, there was no any infection of *B. oryzae* under both storage condition viz. normal (Tem 18-40°C, RH 40%-98%) and dehumidified (Tem 18±2°C, RH 40%) as well as in both containers till five years.

Acknowledgements

The authors would like to acknowledge with deep appreciation and gratitude to Management of Supreme Seed Company Limited for giving her opportunity to conduct the research.

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