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Range of various fungal infections to local and hybrid varieties of non-germinated lentil seed in Bangladesh

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ABSTRACT

To estimate fungal infection range in local and hybrid lentil seed varieties in Bangladesh this study was implemented stepwise. After collecting healthy and qualified lentil seeds from different location of Bangladesh, purity test was scrutinized as well as germination capability of seeds was also checked through blotter incubation method. Non-germinated seeds were then observed under stereo binocular microscope firstly and then on sophisticated camera attached light microscope to identify which fungus species infected them. 15 local and 15 hybrid varieties were taken and there were different varieties of fungal infection happened. Five of the fungal species infected local varieties whereas five species attacked hybrid varieties. As Bangladesh is agriculture depended country and lentil is one of the important crops in our country, farmers will be awarded of which fungal varieties are responsible for lentil seed infection through this study and can increase lentil production by applying appropriate bio-fertilizer against specific fungal species.

Key Words: *Lentil seed, Local and Hybrid varieties, Germination capability and Fungi infection*

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I. Introduction

Lentil, scientifically named as *"Lens culinaris"*, is a precious as well as one of the most staple sources of protein. By holding unique position in the world's agriculture Lentil acts as the oldest crop of the world since last 8000 years (Satter *et al.,*1996). But low yield of lentil is a matter of regret. The yield of lentil in Bangladesh is low in comparison to Syria, Canada, U.S.A, and Ethiopia several strains of fungi are engaged in decaying the consistency of lentil through surviving in seeds and in the residues of lentil for a long time and gets ready to infect the next generation. Corn and other cereals are affected under fairly high moisture. Some minor but realistic recommendation for the control of plant disease was reported in the writing of ancient Greeks Homer (1000BC), Democritus (470BC), and

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Theophrastus (300BC). There are about 7000-1.5 million species of fungi while most are soil borne. When the synthetic fungicide used in 1970s, the new isolates of fungi appeared with their resistance to fungi. Bio-agents or botanical agents can be applied to control seed born pathogen (Agrios, G. N., 2006). A variety of fungi can cause serious damage to the lentil specifically in the seeds namely *Macrophomina phascolina* (for dry root), *Fusarium orthoceros var. lentidis*, *Uromyces fabae* (for rust), Bean Yellow Mosaic Virus (for yellow mosaic). The control of the fungus in lentil is a very difficult task (Ashrafuzzaman, 2006). BADC (Bangladesh Agricultural Development Corporation) supplies only 1.0-1.15% seeds (Fakir *et al.,* 2007). In each year our losses in lentil yield, experienced by the marginal farmers' lies on the action of the pathogens specifically the fungus. The subsequent impact reflects the phenomena of reduced GDP (Gross domestic product) as a remarkable portion of agricultural sectors. For such a developing country like Bangladesh, pest-control-issue might be a big task for the government for lentil production and marketing, where thousands of hectares of lands are utilized for the agriculture purpose. Proper investment in getting new technologies, subsidiary to the lentil farmers and monitoring of pathogenic research on continuous basis would be a better choice for the future of sound production of lentil in our country. Study objectives were to identify and characterize typical microbes responsible for lentil seed spoilage, to analyze the rate of germination for local and hybrid varieties and to establish a contrast in susceptibility between local and hybrid varieties of lentil to the microorganisms.

II. Materials and Methods

Collection of seed sample: The experiments were conducted in vitro in the Plant Pathology Laboratory of Regional Agricultural Research Station of BARI, Jessore. Collected vigor and healthy seeds of local and hybrid varieties of lentil (*Lens culinaris*) from different locations with the assistance of Bangladesh Agricultural Research Institute (BARI), were used in this study. Here, three lentil varieties named BARI-5, BARI-6 and BARI-7 from 15 different locations (Figure 03), where the abbreviations refer the names of the locations) and local varieties from 15 different locations were collected (Figure 02).

Purity analysis: From each sample, 100 seeds were taken following standard procedure seeds were divided into three components viz. pure seeds, other seeds and insert matter. The weight of each component was taken by electric balance.

Germination test: Bottler incubation method was used for germination test. 100 seeds were randomly taken from each sample. The seeds were planted on water soaked two folds blotter in 7.5 cm glass petri dishes, 25 seeds were planted at equal distance. All the petri dishes were incubated at 20-25 degree centigrade (at room temperature). After 7 days of incubation, petri dishes were observed for determining the germinated and non-germinated seeds in naked eye. Data was recorded from each sample.

Microscopic observation: The incubated seeds were observed individually under stereo binocular microscope at 20X to 40X magnification in order to record the incidence of pathogenesis. For further observation a sophisticated camera-fixed light microscope was used where lacto-phenol-cotton-blue dye was used for proper morphology analysis and to take necessary pictures of the microbes.

III. Results and Discussion

Purity analysis

The highest number of pure seed was found from Madaripur (Shibchar-1) and Narail (Kalia-2) had the lowest. The highest amount of other seed was found in the seed sample of Shariatpur (Naria-1) and the lowest percentage of other seed was found in sample of Madaripur (Shibchar-1). Inert matter was maximum in seed sample of Shariatpur and minimum in seed sample from Madaripur (Figure 01). Few locations of Bangladesh contain better soil pH level and standard humidity (10%-14%) while few others are not so up to date for cultivation pattern and environmental interference. According to analyzing all parameters Madaripur is best for apparently healthy seeds.

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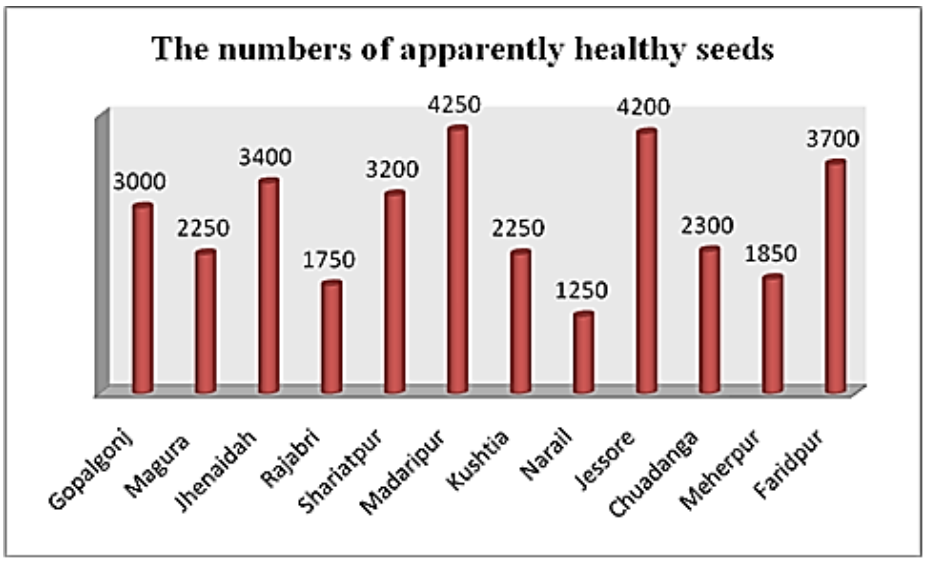

Figure 01.The schematic representation of the quantity of apparently healthy seeds after dry inspection from different locations.

Germination test

After 7 days incubation, the farmer's saved lentil seed samples showed significant differences in percent germination within a range of 48% to 100%. The highest germination was recorded in the seed sample of Kushtia (100%). In case of healthy seedling, the maximum percent of healthy seedling was found in the sample of Kustia and minimum percent of healthy seed was found in sample from Chuadanga. The percent of infected lentil seed range from 40% to 52%. The maximum percent of infected seed (62%) was found in sample of Keshabpur, Jessore and the minimum percent of infected seed (0%) was found in sample from Kustia (Veramara-1). The local varieties of our country were of better germination with 53% to 100% (Figure 02) than that of the hybrids with only 49% to 97% (Figure 03).

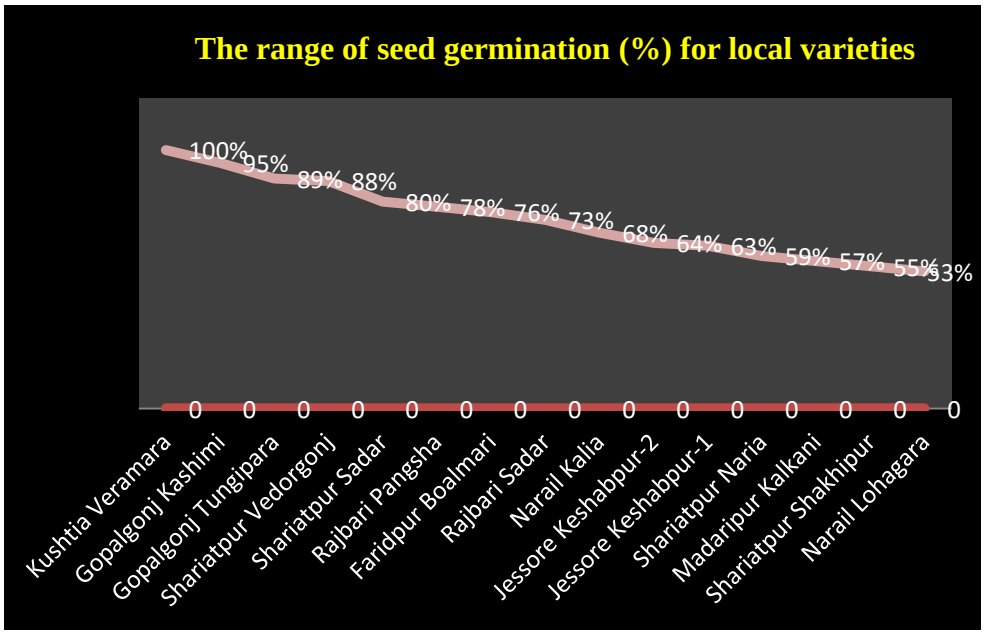

Figure 02. The diagram represents the rate of seed germination of the local varieties where the range varies from 53% to 100% for Narail Lohagara to Kushtia Veramara respectively.

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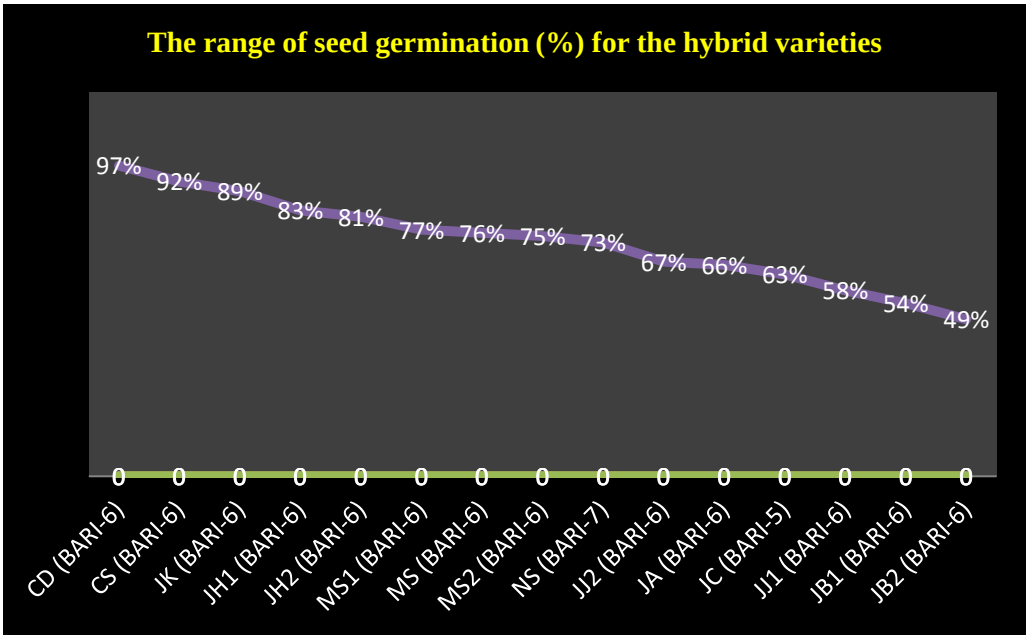

Figure 03. The line graph demonstrates the rate of seed germination for the hybrid varieties, where the range varies from 49% to 97% for JB2 (BARI-6) to CD (BARI-6) respectively.

Microscopic observation

Non-germinated seeds of different local and hybrid varieties were taken under microscope. After morphological studies of various fungal species and matching the characteristic similarity, infection of five fungal species in local lentil seed varieties and five fungal species in hybrid varieties were observed and confirmed. The amount of *Alternaria spp.* and *Fusarium spp.* found in hybrids were double and almost triple respectively than the local varieties. The number of *A. flavus* is 5% more in case of hybrid varieties than the local varieties. The hybrid varieties possessed few positive sides due to having no *Penicillium spp.* where the rate was about 17% for the local varieties. At the same time the amount of *A. niger* was 11% less than the local varieties (Figure 04). Besides, all these items, there was about 0.75% *Stemphyllium spp.* remained non-mentioned in the diagram of the hybrids, but it was not present in the local varieties.

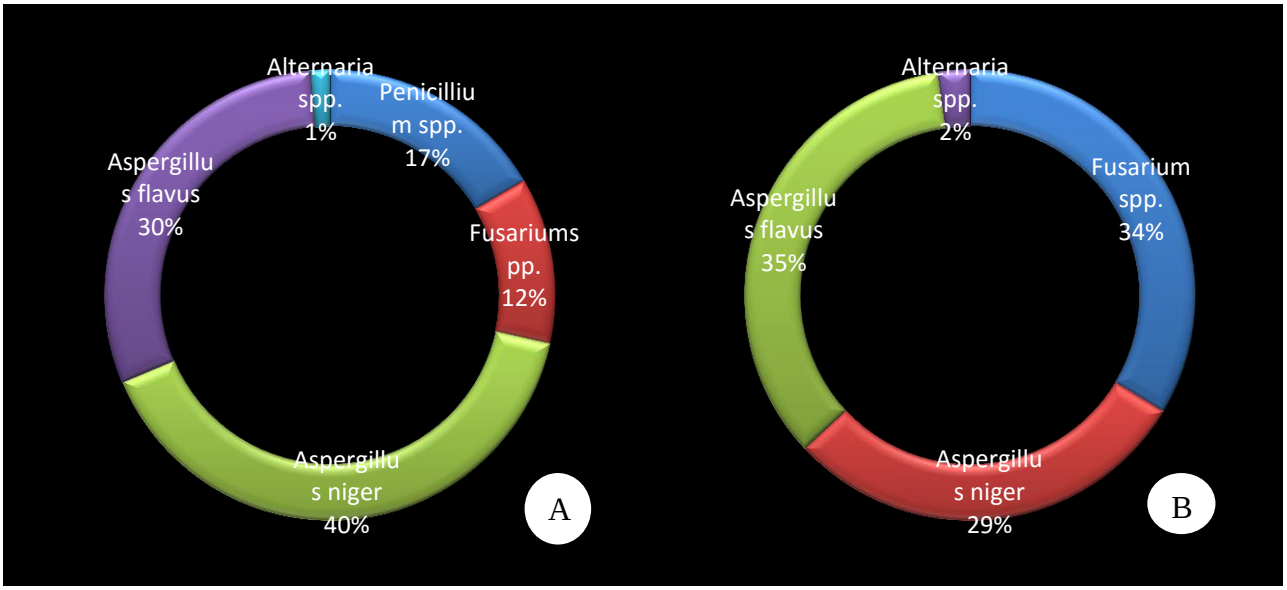

Figure 04. The rate of microbial infection in local (A) and hybrid (B) varieties.

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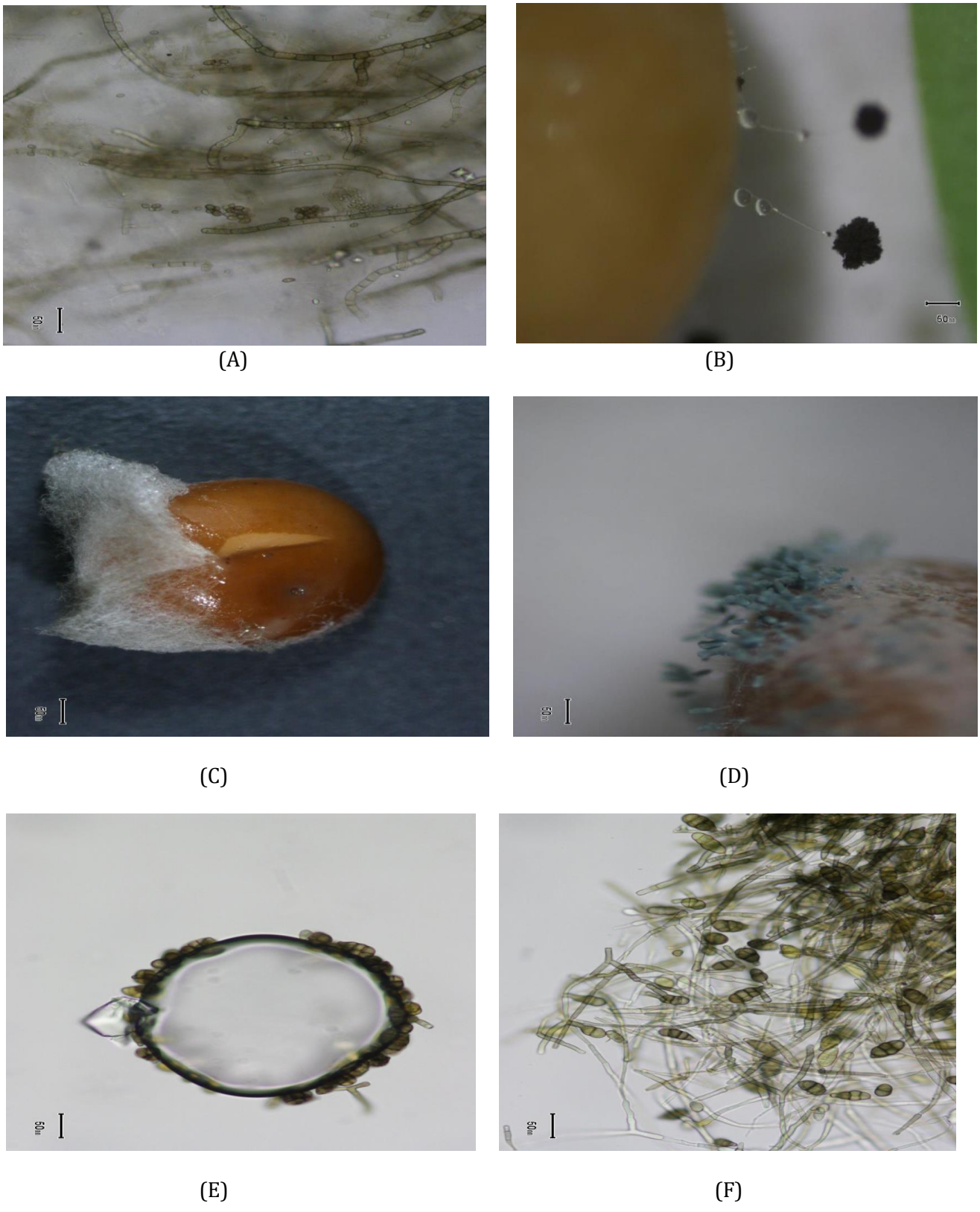

Figure 05. Microscopic observation of various types of fungi from the cultured samples- (A) *Penicillium spp.* (B) *Aspergillus niger* (C) *Fusarium spp.* (D) *Aspergillus flavus* (E) *Alternaria spp.* (F) *Stemphyllium spp*.

Seed borne disease is a serious problem for successful lentil (*Lens culinaris*) production. Among all the group of the seed borne pathogens, fungi play significant role in causing seed borne disease. Some research works have been done to estimate the population and control of seed borne fungi in the seeds and improving lentil seed quality. To analyze the present situation of using local seeds and hybrid varieties, the aforementioned research has taken place. For the estimation of range of fungal infection to various local and hybrid varieties of germinated lentil seeds, lentil seeds sample were collected

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from different location of Bangladesh. Collected healthy and vigor seeds were then undergone purity test, germination test and then observed under microscope. In purity test, we got huge range of pure seeds (30000 to 33000) that was more than 60% of total seeds. Among them, according to location more than 80% of pure seeds were found in Madaripur while Narail containing below 20% of pure seeds. Other seed varieties were almost 40% in Shariatpur and were highest in amount in contrast to lowest amount (less than 5%) in Madaripur. The amount of inert matter was also highest in percentage (more than 30%) in seeds of Shariatpur whereas less than 5% inert material was found in seeds of Madaripur.

Before microscopic observation, germination test was carried out and resulting in 48% to 100% germination where 100% germination and no infected seeds were found in seeds from Kustia. The range of infected lentil seeds varies from 40% to 52% while maximum percentage (62%) was recorded in seeds of Keshobpur, Jessore. Non-germinated seeds are then observed under microscope. Through the morphological characteristics different types of fungi, four species namely *Aspergillus niger*, *Aspergillus flavus*, *Alternaria spp.*, *Fusarium spp.* were found from both local and hybrid seed varieties whereas *Penicillium spp.* and *Stemphyllium spp.* were gotten in local and hybrid seed varieties respectively.

In this study, *A. niger* was observed with its black structure surrounding the samples with spore forming traits. *Fusarium spp.* was seen with its cotton shape structure surrounding the seeds. Gradually it covered the whole seeds restricting the necessary nutrients supply for its germination. *A. flavus* with its bluish-green structure were presented around the seed sample. It was very similar to *A. niger* in morphology except color. *Alternaria spp.* was observed with its mycelium structures surrounding a drop of water. *Stemphyllium spp.* was with its spores and mycelium. *Penicillium spp.* was seen with all of its mycelium and spores (Figure 05).

Microbial pathogenesis is the main precursor of creating infection resulting loss of yield and seed spoilage but it is not the sole to be blamed. Rather there are conditions on the environment suitable to reestablish problems. As Philip and Faye (2009) observed that under the right selection pressure and environmental conditions, new pathogens appear, giving rise to new problems. For example, *Stemphylium* blight of lentil has appeared occasionally in recent years, including 2009, whenever the environmental conditions are right. According to Haque and Khan (2007) research, among the atmospheric factors maximum and minimum temperature, relative humidity ranging from 20.5-29.0 degree centigrade, 5.4-13.3 (mean 14.2) and 78.0-84.5%, 48.0-63.0%(mean 55.5%) respectively are the predisposing factors for disease initiation. Whereas the maximum and minimum temperature, relative humidity ranging from 20.5-35.0 degree centigrade and 13.0-21.0 degree centigrade, 78.0-100.0% and 48.0-80.2% respectively and rainfall 1.2-14.6 mm are favorable for disease development.

At the same time we observed that the contribution of fungi was quite remarkable in most cases in Bangladesh, in the same way Javed *et. al.* (2000) studied on 14 samples of lentil plant parts and seeds in Faisalabad, Pakistan and got 9 different fungi including two well-known pathogens of lentil; *Ascochyta lentis* and *Botrytis cinerea* were recorded on all parts i.e. stem/leaves, pods and seed of the plants in a range of 5-25 and 12.5-45%, respectively. But these two were not common to our local and hybrid varieties to be infected as we mentioned in our research.

It came to our phenomena that *Aspergillus spp.* are the main concern for our most of farmers saved lentil seeds either the local or the hybrid varieties. This condition was familiar to the Indian farmers as well. Singh and Tripathy (1999) carried out an experiment of stored seeds of lentil. They recorded 10 fungal species including *Aspergillus flavous*, *A. niger* etc. were the dominant species as following the experiment on lentil seed to detect seed mycoflora by Jhutha *et al.* (1997) and got similar result.

Fusarium spp. are common in lentil seed losses in some extent but using the natural substances they can be controlled and manipulated, like Arun *et al.* (1995) found that the extract of Garlic bulbs was effective in suppressing redial growth of *Fusarium spp.* and *Sclerotium spp.*

Aspergillus flavus is spore forming like all other *Aspergillus spp.* and can transmit from one row to another very quickly in fields responsible for aflatoxin synthesis as the major food toxic agents.

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Moslem and Parvez (1993) isolated fungi from lentil seeds collected from Riyadh, Saudi Arabia, using the standard blotter and agar plate methods. Of 38 fungal species isolated from lentil seeds, the predominant genera were *Aspergillus* (10 species) and few other. In the same way we got *Aspergillus spp.* with the major responsibility of pathogenesis in lentil seed spoilage in our country for both the local and hybrid varieties.

IV. Conclusion

From the non-germinated lentil seeds of fifteen local and fifteen hybrid varieties, infection of five fungal species to local varieties as well as five fungal species infection to hybrid varieties were observed. Furthermore, 16s rRNA analysis can be carried out for better identification of fungal species.

Acknowledgement

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V. References

[1]. Ashrafuzzaman, H (2006). Udvid Rog Bighan (In Bangla), Publication Bangla Academy, Dhaka-1000, Bangladesh. p. 260.

[2]. Arun, A., Tekha, C. & Chitra, A. (1995). Effect of Allicin and Garlic and Bigonia on two Fungi. *Indian J. Mycol. Plant path.* 25 (3), 316-318.

[3]. Fakir, G. A. & Rahman, G. M. M. (1989). Pulses disease research at BAU: a review. A paper presented in the second national workshop on pulses held at BARI, 6-8 June 1989.

[4]. Agrios, G.N. 2004. Plant pathology. Fifth edition. ELESEVIER, Academic Press, p. 922.

[5]. Haque, M. I. & Khan, A. Z. M. (2007). Effect of sowing dates on the incidence of *Stemphylium* blight of lentil during 1998-2001. *Bangladesh Journal of Scientific and Industrial Research*, 42(3), 341-346.

[6]. Javad, M. S., Wahid, A. & Gill, M. A. (2000). Mycoflora detected from lentil plant and seed in Faisalabad. *Pakistan J Phyropathol.* 12(1), 15-17.

[7]. Jhutha, R., Choudhary, S. L., Jain, K. L., Dhinwa, V. K. & Ram, J. (1997). Effect of culture filtrates of seed Mycloflora of Lentil on seed germination and seedling survival. *L. mycol. and Plant Pathol.* 27(3), 342-343.

[8]. Moslem, M. A. & Parvez, S. (1993). Seed borne fungi of *Lens esculenta*, *Hordium vulgare* and *Tritichum aestivum* from Saudi Arabia. *Intl. J. Trop. Plant Diseases,* 11(1), 99-105.

[9]. Philip, N. & Faye, D. (2009). Effect of sowing dates on the incidence of *Stemphylium* Blight of lentil. *Bangladesh journal of Scientific and Industrial Research*, 42(3), 341-346.

[10]. Satter, M. A., Podder, A. R., Chandra, M. C. & Rahman, M. (1996). The most promising BNF technology for green legume production in Bangladesh. BNF Association, Dhaka, BD. 28, Nov, 1994. Pp. 15-20.

[11]. Singh, J. & Tripathy, S. C. (1999). Mycoflora association with stored seeds of *Lens esculenta*. Herbal Pesticides Lab., Dept. of Botany, Gorakhpur Univ. Gorakhpur, India.

Abbreviations:

CD (Chuadanga Damurhuda), CS (Chuadanga Sadar), JK (Jhenaidah Kaligonj), JH (Jhenaihad Harinakunda), MS1 (Magura Salikha 1), MS2 (Magura Salikha 2), MS (Magura Sreepur), NS (Narail Sadar), JJ (Jessore Jhekargacha), JA(Jessore Avainagar), JC (Jessore Chaugacha), JB (Jessore Bagharpara), BARI (Bangladesh Agricultural Research Institute), BADC (Bangladesh Agricultural Development Corporation)

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