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Identification of Fungi Associated with Different Pigeon Pea Seed Accessions from Iran

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Abstract: A species of *Leguminosae* family is *Cajanus cajan* (L.) Millsp. (Pigeon pea) is an herbaceous, annual, self-pollinated legume pulse crop. The pigeon pea is mainly cultivated in many countries of South Asia due to its nutritious quality and a good source of minerals and protein. Seeds are a common carrier of plant pathogens, which act as the primary source for the distribution of diseases. Contaminated seeds can often create defecated germination and poor seedling vigour. They lose viability much faster than non-contaminated seeds. A large number of mycoflora were reported to be associated with the pigeon pea seeds. To assess the seed health and quality of some pigeon pea accessions this experiment was conducted at the genetic department and national plant gene bank of Iran, seed, and plant improvement institute in 2021. In the experiment, a total of 52 accessions from the pigeon pea collection were used for laboratory tests. During this study different species of *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., and *Rhizopus* spp. were found along with germinating seed and seedlings of contaminated pigeon pea. The most identified species on the contaminated pigeon pea seeds were *Rhizopus* spp., with 50%, and the least were *Alternaria* spp. and *Aspergillus* spp., with 7.2%. The other species *Penicillium* spp., and *Fusarium* spp., were measured on 21.4% and 14.2% of the contaminated seeds respectively. On contaminated pigeon pea seeds, 20 to 100% reduction in seed germination has been measured. Paying attention to seed health is the fundamental strategy for the integrated management of the disease for durable crop production and seed conservation.

Key words: *Cajanus cajan*, *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., *Rhizopus* spp.

İran'dan Farklı Güvercin Bezelye Tohumu Katılımları ile İlişkili Mantarların Tanımlanması

Öz: *Leguminosae* familyasının bir türü *Cajanus cajan* (L.) Millsp'dir. (Güvercin bezelyesi) otsu, yıllık, kendine tozlanan baklagil bitkisidir. Güvercin bezelyesi, besleyici kalitesi ve iyi bir mineral ve protein kaynağı nedeniyle esas olarak Güney Asya'nın birçok ülkesinde yetiştirilmektedir. Tohumlar, hastalıkların yayılması için birincil kaynak görevi gören bitki patojenlerinin ortak bir taşıyıcısıdır. Kirlenmiş tohumlar genellikle dışkılanmış çimlenme ve zayıf fide canlılığı yaratabilir. Kirlenmemiş tohumlardan çok daha hızlı canlılık kaybederler. Çok sayıda

mikofloranın güvercin bezelye tohumlarıyla ilişkili olduğu bildirildi. Bazı güvercin bezelye katılımlarının tohum sağlığını ve kalitesini değerlendirmek için, bu deney İran'ın genetik departmanı ve ulusal bitki gen bankası, tohum ve bitki geliştirme enstitüsünde 2021 yılında yapılmıştır. Denemede güvercinden toplam 52 katılım yapılmıştır. laboratuvar testleri için bezelye koleksiyonları kullanıldı. Bu çalışma sırasında *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp., Penicillium spp. ve Rhizopus spp.'nin farklı türleri. kontamine güvercin bezelyesinin filizlenen tohumları ve fideleri ile birlikte bulundu. Kontamine güvercin bezelye tohumları üzerinde en fazla tespit edilen tür %50 ile *Rhizopus* spp., en az ise *Alternaria* spp. ve %7.2 ile *Aspergillus* spp. Diğer türler *Penicillium* spp. ve *Fusarium* spp., kontamine tohumların sırasıyla %21.4 ve %14.2'sinde ölçülmüştür. Kirlenmiş güvercin bezelye tohumlarında, tohum çimlenmesinde %20 ila %100 azalma ölçülmüştür. Tohum sağlığına dikkat etmek, sürdürülebilir mahsul üretimi ve tohumların korunması için hastalığın entegre yönetimi için temel stratejidir.

Anahtar kelimeler: *Cajanus cajan*, *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., *Rhizopus* spp.

Introduction

Pigeon pea (*Cajanus cajan* (L.) Millspaugh, 2n = 2x = 22) is the sixth most important leguminous crop in the world and an essential food crop in tropical and subtropical regions of the world. Pigeon pea is known as red gram, tur, or arhar. This is a multipurpose product cultivated mainly due to its edible seed, which is rich in protein (up to more than 31%) and essential amino acids such as leucine (16.148 g/kg), tyrosine (14.77 g/kg), and arginine (13.51 g/kg), also contain lipids (3.20%), soluble sugar (5.10%), starch (48.4%) and also crude fibre (8.2%), iron, sulphur, calcium, potassium, manganese, and water-soluble vitamin (Chaudhari et al., 2017; Ade-Omowaye. et al., 2015)

There are numbers of reports about fungi linked to pigeon peas during storage that deteriorated the quality of the seed. The quality and quantity of seeds, depend on seed health which is affected by various types of fungi called seed mycoflora or seed-borne fungi. Scientists found that pigeon pea seeds were contaminated by fungi during harvest, transport, processing, and under storage (Patil et al., 2012). There are reports about some fungi that could cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity, as well as seedling damage (Jalander and Gachande, 2011). Various seed borne fungi affect pigeon pea leading to drop in quality and amount of the seed. Texture, shape and color of the seed that indicate decline of seed health. Contaminated seeds can often create defecated germination and poor seedling vigour. They lose viability much faster than non-contaminated seeds.

To identify fungi associated with seed of pigeon pea accessions and for monitoring the pigeon pea collection seed health and quality this experiment was conducted at the genetic department and national plant gene bank of Iran, seed, and plant improvement institute.

Material and Methods

To assess the seed health and quality the accessions under regeneration from pigeon pea collection of the national plant gene bank of Iran during 2021 were selected. The isolation of the seed-borne fungi associated with the pigeon pea seed samples used the blotter paper method, 10 seeds per Petri plate, was surface sterilized for two minutes with 1% sodium hypochlorite solution followed by three subsequent washings in sterilized distilled water and non-surface sterilized seed, was placed at equal distance on three layers of properly moistened sterilized blotters. These plates were incubated at a temperature of 25±2 °C for 12 h in alternating cycles of light and darkness. The seeds were examined regularly for the growth of fungi (ISTA, 2022).

The fungi were purified on PDA (Potato Dextrose Agar) medium and were identified after seventh day. The Ogawa binocular compound microscope with a magnification of 10X, 40X and 100X. was utilized to determine fungus genera in each plate. The fungus genera were identified based on colony character, texture, color, and sporulation. The seed-borne fungi were identified using identification keys and cross-checked for each seed plate to identify the kind of fungus growing on each seed, Frequency of fungi isolates presence was calculated by applying the following formula: PF = (No. of seeds on which fungus appears / Total number of seeds) × 100. All data was then entered into a Microsoft Excel spreadsheet for analysis that

Frequency of fungi isolates species presence was calculated.

Results

During this study regenerating accessions of Pigeon pea collection were studied at the genetic department and national plant gene bank of Iran, Seed and Plant Improvement Institute in 2021.

One of the key findings of this task Base on the blotter method study was that the most identified species on the contaminated pigeon pea seeds were *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., and *Rhizopus* spp. (fig 1). *Rhizopus* spp., with 50%, *Alternaria* spp. and *Aspergillus* spp., with 7.2%. the other species *Penicillium* spp., and *Fusarium* spp., were measured on 21.4% and 14.2% of the contaminated seeds respectively.

One of the most significant findings in the paper was a 20 to 100% reduction in seed germination that has been measured on contaminated pigeon pea seeds. The results of this analysis are summarized in table 1. There was not any infection on other accessions

Discussions

Based on result of this project *Rhizopus* spp., *Alternaria* spp. and *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp., identified on the contaminated pigeon pea seeds. Kandhare (2014) demonstrated the following seed-borne fungi on pigeon pea seeds: *Aspergillus flavus*, *Drechslera tetramera, Aspergillus nidulans, A. niger, Curvularia lunata, Cladosporium spp., Chaetomium globosum, Colletotrichum truncatum* and R*hizopus stolonifer*. Patil et al. (2012) denoted that appearing fungi on pigeon pea seeds were *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Botrytis cinerea., Chaetomium globosum, Cladosporium herbarum, Curvularia lunata, Fusarium oxysporum, Fusarium moniliforme, Fusareum roseum, Macrophomina phaseolina*, *Penicillium notatum, Phytophthora* sp., *Rhizoctonia solani* and R*hizopus stolonifer*.

Some fungi on contaminated seeds have a great effect on germination. The major effect is related to *Rhizopus* spp, which prevents seed germination in many cases. Chaudhari et al., (2017) and Patil et al. (2012) warned seed borne fungi could be primarily affecting seed viability. This issue can be related to the production of mycotoxins produced by these fungi. Chaudhari et al., (2017) indicated that Seed borne fungi cause qualitative and quantitative losses during storage and adverse effect on seed quality by decreasing seed protein and lipid content.

Cauton should be taken with generalizing the findings to this report as the most identified species on the contaminated seed of pigeon pea is related to the collection location and the condition of the field for pigeon pea regeneration. It seems that attention to the type and collection origin of accessions in the future study could help to determine the fungi species that are most prevalent in contaminated seeds that after a series of investigations could lead us to the susceptibility of pigeon pea seed to that species of fungi. It could help to find and develop practices and provide conditions to restore better seed health.

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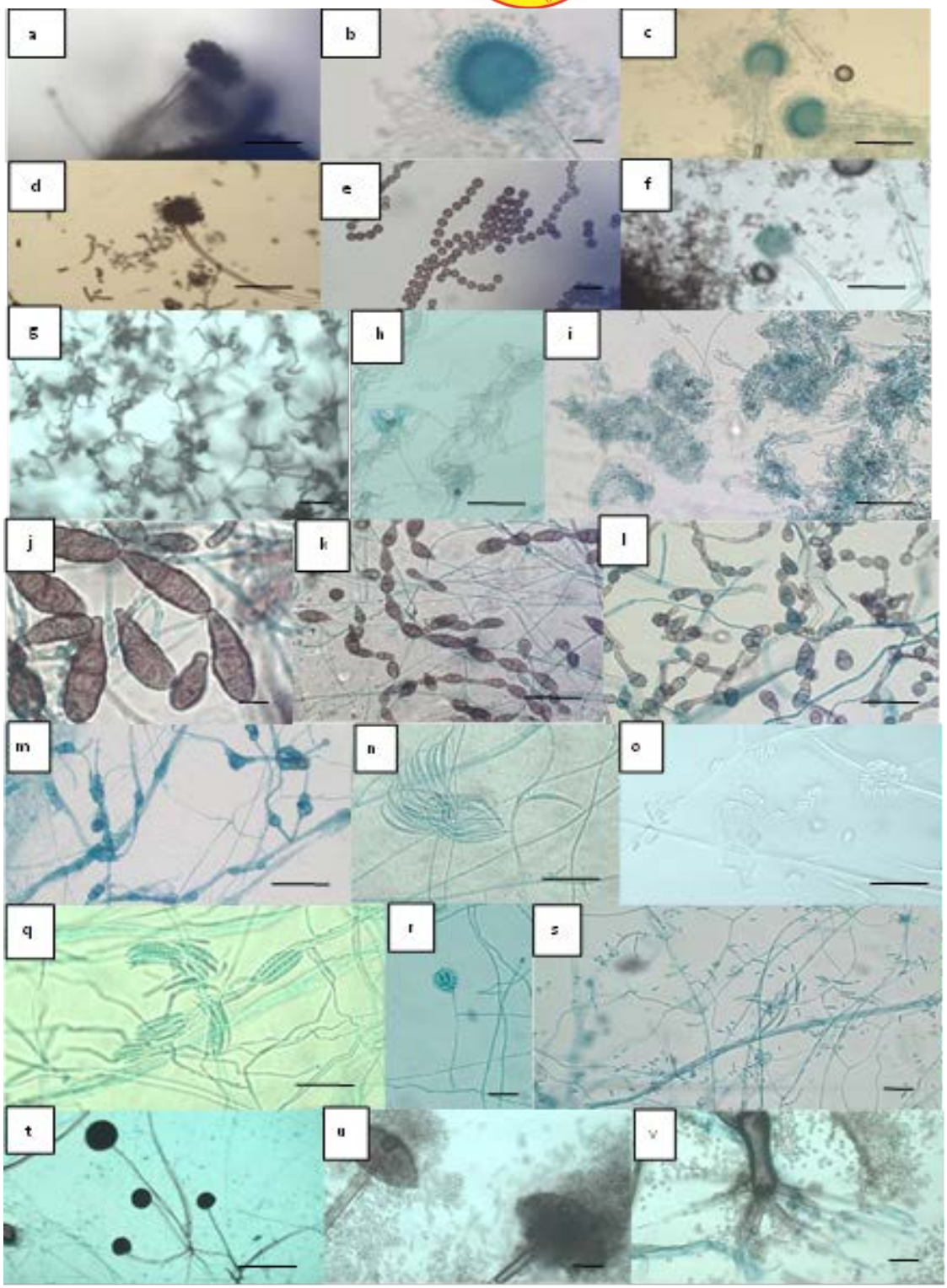

Figure 1. The identified fungi species on the contaminated pigeon pea seeds: a, b, c, d, e, f= *Aspergillus* spp.; g, h, I, = *Penicillium* spp.; j, k, l, = *Alternaria* spp.; m, n, o, q, r, s, = *Fusarium* spp.; t, u, v, = *Rhizopus* spp. Scale bar for a, c, d, f, h, i, k, l, m, n, o, q, t = 50 µm; b, j, u, v= 20 µm; g, r, s = 30 µm

Table 1. % Germination and fungal genus identified on contaminated pigeon pea seeds

TN/no	% Germination	*Alternaria* spp.	*Fusarium* spp.	*Penicillium* spp.	*Rhizopus* spp.	*Aspergillus* spp.
69-00	80		+			
79-00	60			+		
73-00	0			+	+	
68-00	30			+	+	
72-00	0				+	
80-00	0				+	
63-80	0				+	
74-00	10		+	+	+	
83-00	40				+	
87-00	50		+	+	+	+
93-00	10	+			+	+
81-00	0				+	
91-00	0				+	
97-00	20				+	
100-00	0				+	
62-91	0			+	+	
62-100	0				+	
92-97	20	+				
62-81	0				+	
62-93	10		+	+	+	
62-87	50		+	+	+	+
62-83	40			+		

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