

Isolation Of Seed Mycoflora Of Cowpea Seeds

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Abstract: Present study was conducted to isolate seed mycoflora of Cowpea seeds (*Vigna unguiculata*), which belongs to Fabaceae family. It grows as agriculturally primary food grain, livestock forage and soil enhancing green manure. It contains a more protein material than any other vegetable product. Two types of cowpea varieties i.e white cowpea variety and brown cowpea variety has taken to study different types of fungi associated with cowpea. The seed samples were collected from Tarnam village, Adilabad district, Telangana. Seed mycoflora was isolated by employing Blotter paper method and Agar plate method. Higher seed borne fungi was isolated from agar plate method. There were thirty species, sixteen genera isolated from these cowpea varieties. The identified genera of fungi were *Alternaria* spp, *Aspergillus* spp *Chaetomium* spp, *Cladosporium* spp, *Drechslera* spp, *Fusarium* spp, *Helminthosporium* spp, *Humicola* spp, *Mucor* spp, *Nigrospora* *Phoma* spp, *Phythium* spp, *Penicillium* spp, *Rhizopus* spp, *Trichoderma* spp, *Trichothecium* spp, *Verticillium* spp. The most common fungi in two varieties of cowpea are *Aspergillus* spp, *Fusarium* spp, *Pencillium* spp, *Rhizopus* spp. The study found white cowpea has a more fungal effect than brown cowpea.

Keywords: Cowpea, Seed mycoflora Agar plate method, Blotter paper method,

1. INTRODUCTION

Cowpea (*vigna unigulata* (L.) Walp) is a plant which belongs to the family Fabaceae. It is now grown worldwide especially in the tropical countries. It is a warm seasonal crop and produced in semi-arid regions. It was the most commonly cultivated, bean used for human consumption in old word. Cow pea is widely popular for the adequate levels of protein (Allen, 1981). It is commonly known as black-eyed pea and southern pea. It is also grown as a vegetable garden plant in most parts of the countries. It also improves soil nitrogen levels and also used as a fodder for animals. The seed contain 24% crude protein, 53% carbohydrates, and 2% fat (FAO, 2012). The seeds serve as a major source of minerals (phosphours, potassium, calcium and magnesium and vitamins (vitamin A and C, thiamine (B1), riboflavin (B2), niacin (B3), pyridoxin (B6) and pantothenic acid (B5)) (Jenkins et al., 2000). The daily intake of cowpeas emphasizes the spleen, within improvement in the cell manufacture with promotes immune system. The seeds are used to treat common cold, warms in the stomach, blood in urine (Van Wyk et al., 2000). They also hold diuretic and anthelmintic properties and used to treat liver complaint associate with the jaundice (Noorwala et al., 1995). As food poppy seeds are eaten in different forms, they could be boiled, fried, roasted mixed with sauce and consumed directly. (Siddhuraju and Becker, 2007).

The cowpea is grown under two varieties namely white cowpea and brown varieties. The main difference between in white cowpea and brown cowpea varieties, the brown cowpeas presence are high minerals are calcium, potassium, and zinc, high protein while white cowpea contains higher carbohydrate and higher fiber, minerals are magnesium, manganese and sodium. But both different seeds have same iron levels (Alayande et al., 2012). Fungal infestation of cowpea seed is a major issue, resulting in decreased quality and nutritional value and toxic to human consumption for cattle feed (Castillo et al., 2004). Many fungal genera; *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Penicillium*, *Rhizopus* and *Trichoderma* known to invade stored grains and pulses (Schafer and Kotanen, 2003). *A. flavus* found to affect lipids and carbohydrates contents of plant seeds in addition to the aflatoxin production (Aziz and Mahrous, 2004). According to Soil Resource Mapping (2012) report 13.39% shrubs cultivating culture is exist sting in Adilabad District. The purpose of this work was to isolate storage fungi associated with cowpea varieties. Cowpea seed samples were collected from Tarnam village, Adilabad district. For identification of seed mycoflora during storage conditions.

2. MATERIAL AND METHODS:

2.1 Collection of Seed Samples

The seed samples were collected from different cultivars of Tarnam village, Adilabad district, Telangana, India. The collected seeds were stored in cloth bags for six months at room temperature for the study of storage fungi.

2.2 Blotter Paper Method

100 seeds were sown on three layers of pre-soaked moist blotter paper with a 9 cm diameter. 25 seeds were placed in each plate into two layers (De Tempe, 1953)

2.3. Agar Plate Method

Agar plate method is commonly used in plant pathogenic research because it provides a nutrient-rich substrate for mycelia growth and pathogen sporulation on seed, especially for slow-growing fungi. PDA media was autoclaved at 1.04 kg/cm² for 15 minutes and heated media (45⁰C) was put into each plate. After solidification, 100 seeds were plated, with 25 seeds on per plate.

Four replicates of 100 seeds per treatment were surfaced sterilized in 1% sodium hypo chloride for 1 minute and then rinsed in several changes of sterile distilled water. Petri plates were lined with two layers of filter papers that had been soaked in distilled water. Five of these Petri dishes were plated with 25 seeds each to represent treatment replicates, and they were arranged in a completely randomized pattern. The Petri dishes were incubated at 20-22°C for 7 days and after 7 days of incubation on Potato Dextrose Agar (PDA), which was prepared according to the manufacturer's instructions.

2.4. Identification of Fungi

The fungal colonies growth was investigated every day by visual, morphological, microscopic and colony appearance by using recommendations given by (K. H. Domsch, W. Gams and T. H. Anderson, 1980).

3. RESULTS AND DISCUSSION:

Seed borne pathogenic fungi in cowpea used for seeds reduce germination, emergence, growth and yield, whereas in cowpea used for food they can reduce the nutritional value or produce toxins making the cowpea unsuitable for consumption. The fungi can be transmitted as contaminants that adhere to the seed coat, or infect the seed, which is considered as the main mechanism of seed-mediated transmission. In this study significant number of fungi was isolated from these seed samples of *Vigna unguiculata*, seeds regularly subjected to deterioration caused by seed borne mycoflora. These fungi imply an irreversible degenerative change in the quality of seed. 10 fungal species with 9 genera were isolated from the table.1 *Alternaria alternate*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Chaetomium spp*, *Cladosporium spp*, *Drechslera spp*, *Fusarium meniliforame*, *Fusarium oxysperium*, *Mucor spp*, *Pencillium chrysogenum*, *Pencillium notatum*, *Rhizopus spp*, and *Trichoderma album*. The most prevalent genera were *Alternaria spp*, *Aspergillus spp*, *Chaetomium spp*, *Cladosporium spp*, *Drechslera spp*, *Fusarium spp*, *Mucor spp*, *Pencillium spp*, *Rhizopus spp*, *Trichodrma spp* were dominant species isolated from the two methods. The following table 1 conforms white cowpea fungal species high dominant than brown cowpea fungal speices. The highest percentage fungal infection was associated with white cowpea seeds (100%) recorded. Among the two methods in Agar plate method most of the fungi were isolated followed by Blotter paper method. The most significant input for crop production is seed, Pathogen-free, healthy seed is critical for optimal plant populations and a satisfactory harvest (Ghangaokar et al., 2013). Many plant diseases are seed-borne, which can result in massive crop losses, as well as a reduction in plant development and agricultural output (Kubiak and Korbas, 1999). Mycoflora found in seeds is significant in regulating seed quality and lifetime. Microbial invasions can cause seed rotting and a decrease of viability, vigor, germination, and oil quality (Krishnappa and Nagaraja 2009). The variation in seed mycoflora was detected from the newly stage to the conclusion of the storage period. Storage fungus were observed in low concentrations in freshly harvested seed samples and were more prevalent as storage time increased. Storage fungus necessitate high osmotic pressure and a lack of water (Manoharachary and Kunwar, 2006).

Plate 1: A= White cowpea, B=Brown cowpea



Table.1 Fungi isolated from the varieties White and Brown Cowpeaseeds

S. NO	Mycoflora	Cowpea			
		White Cowpea		Brown Cowpea	
		Un	T	Un	T
1	<i>Alternaria alternate</i>	+	+	+	+
2	<i>Aspergillus flavus</i>	+	-	+	-
3	<i>Aspergillus flavipes</i>	+	+	+	+
4	<i>Aspergillus humicola</i>	+	+	+	+
5	<i>Aspergillus fumigatus</i>	+	-	+	+
6	<i>Aspergillus niger</i>	+	-	+	-
7	<i>Aspergillus nidulans</i>	+	+	+	+
8	<i>Chaetomium herbarum</i>	+	+	+	+
9	<i>Cladosporium sp</i>	+	-	+	-
10	<i>Drechslera halodes</i>	-	+	-	+
11	<i>Fusarium meniliforame</i>	+	-	+	-
12	<i>Fusarium oxysperium</i>	+	+	-	+
13	<i>Fusarium roseum</i>	+	-	+	+
14	<i>Mucor sp</i>	-	+	-	+
15	<i>Nigrospora spp</i>	-	-	+	+
16	<i>Helminthosporium sp</i>	+	+	-	+
17	<i>Humicola sp</i>	-	+	-	+
18	<i>Penicillium chrysogenum</i>	-	+	-	+
19	<i>Penicillium citrinum</i>	+	+	+	+
20	<i>Penicillium notatum</i>	+	+	+	+
21	<i>Phoma sp</i>	+	-	+	-
22	<i>Phythium sp</i>	-	+	-	+
23	<i>Rhizopus nigricans</i>	+	+	+	+
24	<i>Rhizopus stolonifera</i>	+	-	+	-
25	<i>Sclerotium sp</i>	-	+	+	+
26	<i>Trichoderma album</i>	+	-	+	+
27	<i>Trichothecium album</i>	+	+	-	+
28	<i>Trichothecium roseum</i>	-	+	+	+
29	<i>Trichoderma viridae</i>	+	+	-	+
30	<i>Verticillium sp</i>	+	-	-	+

Un = Untreated (Seeds were without sterilized), **T** = Treated (seeds were surfaced sterilized in 1% sodium hypo chloride for 1 minute and then rinsed in several changes of sterile distilled water), + = Present, - = Absent

4. CONCLUSION

In conclusion, in the present investigation cowpea associated mycoflora were isolated by the two-method blotter paper method and agar plate method. Among them Agar plate method had recorded more fungi than the other method. *Vigna unguiculata* seeds regularly subjected to deterioration caused by seed borne mycoflora. These fungi imply an irreversible degenerative

change in the quality of seed, vigour index and germination capacity. The most prevalent genera were *Alternaria* spp, *Aspergillus* spp, *Cheatomium* spp, *Cladosporium* spp, *Drechslera* spp, *Fusarium* spp, *Mucor* spp, *Pencillium* spp, *Rhizopus* spp, *Trichodrma* spp were dominant species isolated from the two methods. The highest percentage fungal infection was associated with white cowpea seeds (100%) recorded, followed by brown cowpea seeds (96.16%).

5. REFERENCES

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