Comparison Of Different Methods To Test Seed Health In Cotton Cultivars

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Abstract: Cotton is susceptible to many diseases. Various pathogenic fungal species are present on its seed surface and their identification is vital step to avoid potential losses. A total of 50 samples of 10 genetically different cultivars viz MNH-93, BH-147, RH-112, CIM-443, CIM-473, BH-118, MNH-552, NIAB-78, CIM-482, MNH-554 were collected from three different districts of Punjab. Two different seed health test methods were compared for their efficacy as seed health test against all the 10 genetically different cultivars. A total of 13 fungi were isolated by blotter paper method from 10 genetically different cultivars. These fungi were *Aspergillus flavus, Aspergillus nigar, Alternaria alternata Cephalosporium spp., Curvularia lunata, Drechslera tetranera, Fusarium moniliforme, Fusarium oxysporum, Fusarium solani, Alternaria alternate, Rhizopus spp., Penecillium spp.,* and *Rhizopus stolonifer, Trichoderma harzianum*. It is concluded from two seed health tests studies; blotter paper test is the best because the maximum number of seed borne pathogens were isolated by blotter paper method in less time and cost and percentage of isolation in variation was reached to 72 %. The analysis showed that cultivars CIM-443 is susceptible due to maximum number fungal species presence. Percentage of isolation was also found to be higher than all other varieties. It is concluded that cotton seed irrespective of variety, should be tested for their health status before sowing and treated to reduce the primary infection due to various seed-borne fungi.

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Introduction

Cotton (*Gossypium hirsutum*) belongs to family Malvaceae (Bombacaceae), is the first important staple fiber, fuel, and food crop of Pakistan. It is also called as white gold and the fiber is most often spun into yarn or thread and used to make a soft, breathable textile. The most widely cultivated species of the world is *Gossypium hirsutum* and it is the leading fiber crop grown in more than 80 countries (Shakeel et al., 2011). The use of cotton for fabric is known to date to prehistoric times; fragments of cotton fabric dated from 5000 BC have been excavated in Mexico and between 6000 BC and 5000 BC in the Indus Valley Civilization. It was originated 7,000 years ago humans started growing cotton in the Indus Valley while 2,000 years later it was cultivated in Mexico and Peru. Fragments of cotton fabric from 5,000 BC have been excavated in Mexico and Pakistan. Since about 6000 BC, this crop has been present in Pakistan in a domesticated form and most of the biodiversity of this species occurs in this country. Pakistan is the fourth largest producer of cotton after China, India, and the USA that has good fiber quality. However, due to some limiting factors including both biotic and abiotic stresses; its production has remained stagnant for the last two decades (Saeed et al., 2014). Cotton spread to east from Pakistan into China, Japan and Korea and west into Europe, reaching Spain in the 900s. it was cultivated on 29.7 million hectares worldwide with the production of 24.5million tons bales (PBS, 2015).1.5 % of GDP while in Pakistan 2961 thousand hectares were cultivated and 13.983 million tons bales production recorded (PBS, 2015). It contains potassium 135 mg (3%), magnesium 11%, Iron 2% (2000 calories) and proteins like vitamin C (1%) and Vitamin B-6 (5%) (Bertrand et al., 2005).

A number of soil borne and seed borne fungi can infect cotton seedling individually or association as disease complex. A wide range of fungi may participate in the process of seed deterioration but a few of these fungi cause pre and post emergence damping off in seedlings. Seed deterioration refers to the breakdown of the cotyledon and embryo tissue within the seed (Kirkpatrik and Rotrock, 2001).

If the fungi have been virulent in seedling, germination can be delayed or may not occur and species of fungi, includes A. alternata, A. niger, Fusarium solani,, Rhizopus arrhizus, Rhizoctonia solani, Penicillium spp. are the causal agent of seed decay and pre emergence damping off (Arabsalmani, 2012). The current losses of yield in the world are about 1.5 million bales. Estimates of the overall

yearly loss recorded were 10 to 15% (Arabsalmani et al., 2004).

Seed is a living tissue and its viability is affected directly by seed moisture contents and temperature during storage. When moisture contents are high (above 16 %), seeds deteriorate very rapidly due to activities of seed-borne microorganisms and sometimes by the attack of insects. The activity and multiplication of storage molds are generally favored by warm moist weather. In Pakistan, various fungi attack the cotton crop such as Aspergillus spp., Alternaria spp., Fusarium moniliforme, Fusarium oxysporum, Fusarium semitecttum, Fusarium solani, Helminthosporium app., Mucor spp., Rhizoctonia spp. (Bhutta and Mathur, 1989 and Hussain et al. 1981). These fungi reduce the yield but also spoil fiber, reduce the oil contents in seed, and adversely affect seed germination. Moreover, the emerging seedling from diseased seeds is weak and results in the poor standard of the crop (Cramer, 1967 and Kamal, 1972).

Detection of seed-borne pathogens is an important factor for the determination of the quality of seeds to ensure health and vigor of the crop. Therefore, proper identification of seed-borne pathogens is a fundamental step in planning management strategies. Two the most important seed health testing methods, viz. the blotter and agar plate methods, have been reported to be simple and suitable for routine seed health testing for a number of pathogens in different crop seeds (Neergaard, 1977).

Keeping in view, the importance of cotton, the present studies were undertaking with the objectives to determine the fungi associated with cotton seeds, their distribution in commercially grown cotton cultivars in main cotton growing districts of Punjab to compare test techniques and to evaluate the efficiency of different fungicides.

Materials And Methods

The following procedures were adopted to carry out the research work on the following lines:

Collection Of Seed Samples

Seeds of ten genetically different cotton cultivars i.e. MNH-93, MNH-552, MNH-554, BH-147, BH-118, NAIB-78, CIM-482, CIM-473, RH-112, CIM-443 were collected from three different research stations like Central Cotton Research Institute, Multan; Cotton Research Station; Bahawalpur; and Cotton Research Station, Rahim Yar Khan.

Comparison of different seed health test methods

Two different seed health testing methods e.g. blotter paper method and agar plate test were used to compare percent seed germination and percent recovery of seed-borne fungi against 10 different cotton cultivars. Following procedure was adopted for each test.

The Blotter Paper Method

Seeds were placed in sterilized Petri dishes on three layers of moistened absorbent paper (blotter). These papers were used to provide enough moisture throughout the duration of the test. In each Petri dish, ten seeds were placed at a proper distance, which were incubated at a temperature of 25 ± 2^0 C for seven days. The seeds used in the experiment were disinfected with 0.1 % mercuric chloride for two minutes. Inert material and pieces of plant debris were removed during the examination of dry seed. A light and darkness cycles of 12:12 hours were provided as a standard procedure (ISTA, 1985). Data was recorded after seven days of incubation through examination of seeds under stereoscopic microscope and by making the studies for the fungal growth.

The Agar Plate Test

The seeds were disinfected with 0.1 % mercuric chloride for two minutes. Then ten seeds were placed in each sterilized Petri dish containing sterilized Potato dextrose agar (PDA) at the proper distance. The Petri plates containing seeds were incubated for seven days at the temperature of 25 ± 2^0 C providing 12:12 hours alternate light and darkness throughout the incubation period. Data on the fungal growth was recorded by preparing the slides from the colonies and by examining the seed samples under the stereoscopic microscope.

Results And Discussion

Comparison of different seed health test methods

Two different seed health test methods such as blotter paper method and agar plate test were used to compare percentage seed germination and percentage recovery of seed-borne fungi against ten different cotton cultivars. Among the two-seed health test studied, blotter paper test was found to be the best (table-1) because the maximum number of seed borne pathogens were isolated by blotter paper method (Bhutta, 1998). The percentage of isolation varied from 0.5 to 72 % (table-2).

Comparison of percent occurrence of mycoflora on different cotton cultivars by blotter paper method

The most efficient method i.e. blotter paper method was used to evaluate seed health of 10 genetically different cotton cultivars on the basis of seed borne fungi were determined. The occurrence of seed borne fungi on different cotton cultivars is given in (table-13) NIAB-78 cotton cultivar proved to be highly resistant to all 13 seed-borne fungi. These isolated fungi ranged from 0.25-16.5 % (Table-13) out of 400 seeds used. The percentage of isolated fungi varied from 0.5-72 % (Table-13). The other varieties showed higher isolation percentage of seed

borne fungi. The analysis showed that cultivars CIM-443 was almost susceptible from which the maximum number of fungi were isolated (table-13). Percentage of isolation was also found to be higher than all other varieties.

Table 1: Comparison of percent occurrence of mycoflora on RH-112 seeds (Cotton cultivar) by using different seed health tests

Fungi Recorded	Blotter paper method	Agar plate test
	P	P
Aspergillus niger	34.75	30
Aspergillus flavus	26.75	24
Alternaria alternate	20	18
Curvularia lunata	1.75	1.0
Cephalosporium spp.	2.75	2.0
Drecheslera tetramera	2.0	2.0
F. moniliforme	3.0	1.0
F. oxysporum	3.75	3.0
F. semitectum	10	7.0
F. solani	3.25	2.0
Penecillium spp.	3.5	3.0
Rhizopus stolonifera	33	28
Trichoderma harzianum	21	19

P= Present

Table 2: ***Percent occurrence of seed borne fungi on cotton cultivars by using blotter paper method***

Average of five sample in each cultivar										
Fungi recorded	***MNH-93***	***NIAB-78***	***BH-147***	***RH-112***	***CIM-443***	***CIM-482***	***MNH-552***	***MNH-554***	***CIM-473***	***BH-118***
Aspergillus niger	***55***	***22.5***	***48.25***	***34.75***	***65.5***	***45.5***	***35***	***64***	***63.75***	***64***
Aspergillus flavus	***44.25***	***16***	***66.75***	***26.75***	***75***	***24.5***	***23***	***52.5***	***43***	***63***
Alternaria alternata	***10.25***	***1.25***	***10***	***20***	***20.5***	***16.5***	***1.5***	***3***	***1.5***	***…..***
Curvularia lunata	***……***	***1.25***	***7***	***1.75***	***8.75***	***13***	***……***	***2***	***1.5***	***7.5***
Cephalosporium spp.	***11.75***	***1.75***	***17***	***2.75***	***14***	***3.5***	***2***	***…..***	***2***	***2.75***
Drecheslera tetramera	***1***	***……***	***4***	***2***	***…..***	***2***	***2***	***3***	***1.5***	***2***
F. moniliforme	***4***	***2***	***3.75***	***3***	***4.5***	***3.5***	***2.5***	***4.25***	***4***	***3***
F. oxysporum	***3.5***	***2***	***3***	***3.75***	***5***	***2.75***	***3***	***3.25***	***2.75***	***2.5***
F. semitectum	***3.25***	***0.75***	***3.75***	***10***	***5***	***6***	***2.75***	***2***	***…..***	***2***
F. solani	***2.5***	***3.5***	***2***	***3.25***	***3***	***2.75***	***2***	***2***	***2.5***	***3***
Penecillium spp.	***6.25***	***2***	***2.5***	***3.5***	***5.5***	***2.25***	***9***	***4.5***	***4***	***5***
Rhizopus stolonifer	***61***	***48***	***21***	***33***	***72***	***18***	***30***	***51.5***	***72***	***50***
Trichoderma harzianum	***9.5***	***8.5***	***18.5***	***21***	***27.5***	***20***	***23***	***22***	***532***	***19***

Identification Of Different Fungi

Identification of the fungi associated with cotton seed was done based on color of colonies, conidiophores, and conidia. These characters of the fungi were compared with available and relevant literature (Raper and Fennell, 1965, Jha, 1993 and Barnett, 1972).

Alternaria alternate Nees ex. Wallr.

Conidiophores dark, mostly simple, rather short, or typically bearing a simple or branched chain of conidia. Conidia: dark, typically with both cross and longitudinal septa. Mycelium septate, branched, light brown in color.

Aspergillus flavus Ling

Colonies were yellow green with white aerial mycelium on edges under a stereomicroscope. Conidiophores were globose and there was 360-65 X 4-6 10µ. Phialides in single series, mostly on the upper half of vesicle. Conidia globose, yellow green, smooth, 2.38-4.76µ in diameter.

Aspergillus niger Tiegham

Colonies were dark brown. Conidiophores were a septate, up to 370µ, long and 8.4-13µ in width. Vesicles, globose, Phialides in two series. Conidia brown to black, smooth, globose, single celled 2.5-3.9µm in diameter.

Cephalosporium spp. Corda

Mycelium: Septate, branched, hyaline. On seeds colonies white. Conidiophores: erect, non-septate, hyaline, arising singly. On seeds conidiophores at right angles from mycelium, bearing conidia at apex in whitish spherical droplets. Conidia: Phialospores produced successively at the apex, usually aggregated in the false heads.

Curvularia lunata (Wakkar and Boedijin)

Mycelium branched, septate, hyphae, hyaline to brown in colour. Conidiophores: septate, erect, curved, sometimes straight and olive brown in colour, 3-septate, apical cell rounded 17.0-35.8µ long and 8-16.4µ wide.

Drechslera tetramera (MCKinney) Subram and Jain

Colonies were dark. Conidiophores: arising singly or in groups of 2-3, brownish in colour. Conidia: mostly cylinderic, ellipsoid, straight with broadly rounded ends, brown in colour, 3-septate, 20.4-35.7 X 6.5-13.6µ.

Fusarium moniliforme Sheldon

Mycelium: yellowish to rose white. On seed, mycelium, purplish or orange coloured scantly or abundant mycelium is present. Micro conidia: produced in chain later, scattered over the mycelium, 1 or 2 –celled, fusiform to avoid, hyaline. Macro conidia: cylindrical, straight or slightly curved, fusiform to falcate, tapering towards either end, scattered or grouped in sporodochia, mostly 3-5 septate, rarely 6-7 septate.

Fusarium oxysporum Shl.

Colonies on PDA were white, mycelium branched, septate, hyaline, bearing chlamydosopre. Conidia borne on Phialides and are of two types: micro and macro conidia. Micro conidia: one or two celled, oval to reiform, 1.2-3.02 X 1.2-1.8µ. Macro conidia: pedicelate, spindle to sickle shaped, 4.03-72 X 1.08-6µ.

F. senitecttum Berk. and Rav.

Mycelium: brownish white in color, on seed yellow colored loose mycelial growth is present on the entire surface of seed. Macro conidia: fusiform to falcate with round apex, mostly 3-septate, 5-septate. Branched conidiophores bearing Macro conidia, is the diagnostic character of this species on seed.

F. solani (Mart). Sacc.

Mycelium: mycelial growth sparse to dense, grayish to white in colour. On seed, abundant dull white growth is present. Micro conidia: abundant, small generally one celled, oblong, straight or curved, hyaline, never found in chain. Macro conidia: cylindrical to falcate, pedicellate at base and with smooth, round apical cell, mostly 3-5 septate, hyaline.

Penicillium spp. Link ex Fries

Mycelium: Profusely branched, septate and hyaline. Conidiophores: Long, erect and septate, which branch two thirds of the way to the tip in broom like fashion. Ultimate branches bearing tufts of flask shaped sterigmata. Conidia: Globose to ovoid borne in chains at the tip of sterigmata.

Rhizopus stolonifer

Mycelium: Well developed, coenocytic with rhizoids. Sporangiophores: Aries from stolons in groups opposite the rhizoids. Sporangia: Spherical blackish with well developed collumella borne terminally and singly on branched sporangiophores. Numerous black pin head like structures develop on mycelium.

Trichoderma harzianum Pers

Whitish green growth of mycelium was observed on the seed under stereomicroscope. Conidiophores were very much branched while conidia were very small and produced in clusters.

Discussion

Like many other crops cotton is prone to attack by many diseases. In Pakistan, various fungi attack the cotton crop such as *Alternaria* spp., *Aspergillus spp*., *Cephalosporium* spp., *Fusarium moniliforme*, *Fusarium oxysporum*, *Fusarium semiectum*, *Fusarium solani*, *Helminthosporium* spp., *Mucor* soo., *Rhizocotonia* spp., and *Rhizopus stolonifer* (Bhutta and Mathur, 1989, Bhutta and Ahmad, 1990).

To know the seed borne mycoflora of ten cotton cultivars, 50 seed samples were collected from the three districts of the Punjab i.e. Bahawalpur, Rahim Yar Khan and Multan. A total of 13 seed borne fungi were detected from ten cotton cultivars but the importance was given to three pathogenic fungi. The three fungi are *Fusarium solani*, *Fusarium moniliforme* and *Fusarium oxysporum*. They are the major problem in most cotton growing areas of the Punjab causing Fusarium wilt and root rot.

Studies on surface sterilization of seed were carried out to know whether the seed borne fungi of different cotton cultivars were surface contamination or internally deep seated. The naturally infected seeds were treated with 0.1 % mercuric chloride. It was concluded that after treatment, the number of seed borne fungi was reduced and their incidence was also decreased which indicated that some fungi were not internally seed borne and were surface contaminants. Disinfection treatment usually suppresses the growth of saprophytic and fast-growing fungi (Bhutta, 1988). Reduction in the seed borne fungi due to disincentive

treatment may suggest that in routine seed health testing, it is not advisable to treat the seed because disinfected seed do not give total recovery of seed borne fungi.

Monitoring of fungal population is a major tool in managing seed borne problems. In this regard, seed health testing methods are fundamental steps in detection and identification of pathogens in any seed certification program to produce disease free quality seed (Bhutta et al, 1992).

Two seed health testing methods viz., blotter paper method and agar plate test were compared for their simplicity and efficiency. (Table-2) out of these two methods, blotter paper method yielded the highest number of fungi, took less number of steps and time and proved less costly as compare to agar plate method (Table-2). However, in present studies almost all the fungi including *Alternaria* spp., *Aspergillus* spp., *Cephalosporium* spp., *Drechslera tetranera*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Fusarium semitectum*, *Fusarium solani*, *Helminthosporium* spp., *Mucor* spp., *Rhizoctonia* spp., and *Rhizopus stolonifer*, *Trichoderma harzianum* were isolated by using blotter paper method which is in accordance with the result of (Bhutta 1988). In present studies, it was noticed that though agar plate method was good as compare to blotter paper method, where these infected seeds were used but pre-treatment of seeds significantly reduced the population of mycoflora associated with cotton seeds. Therefore, pre-treatment may not be advised in testing for seed certification, storage and recommending regarding feeding value of any seed and grain lot. Moreover, agar plate test proved less economical and more time consuming than blotter paper method. It is thus concluded that, in terms of efficiency and accuracy in detection, the blotter paper method is the most appropriate. Seed-borne diseases can be managed efficiently by using diseased free seed to a certain extent. In this case, scientifically based disease standards should be implemented.

However, if to an extent, the infection crosses the economic threshold level and tolerance limit then infection can be minimized by seed treatment with seed dressing fungicides. Seed disinfections with fungicides is one of the most effective and realistic methods of reducing losses caused by various diseases. The method is simple in execution and inexpensive in the application.

In present studies, four available seed dressing fungicides were used Out of four fungicides i.e. Benlate, Topsin M, Diethane M-45 and Vitawax, Benlate proved to be the most effective against pathogenic fungi i.e. Fusarium solani, F. oxysporum and F. moniliforme.

Fuchus et al (1970) reported that the systemic activity of fungicides against F. oxysporum. According their results, Benlate at 1 mg/L in liquid media or agar plate inhibited the mycelial growth. Svampa (1976) noticed during trials on F. oxysporum on tomato that Benomyl and Topsin M at high dose gave moderate control when sprayed after every five days. In our finding Benlate inhibited the mycelial growth of F. oxysporum in laboratory conditions Baicu and Goga (1971) reported that F. oxysporum was strongly inhibited by Topsin M than Benomyl. But in present experiment Benlate was more effective than

Topsin M against *F. oxysporum.*

Our results are in line with the results of Kadous et al (1984) who synthesized 13 compounds which were studied for chemical and physical properties and activities were compared against Rhizoctonia solani and Fusarium oxysporum f. sp. Vasinfectum in vitro and as seed treatment of cotton. Some compounds proved more effective than Captan and Vitawax. Sharma and Bedi (1985) evaluated nine fungicides in wilt sick field for their efficacy in controlling the wilt of cotton caused by Fusarium oxsyporum f. spp. vasinfectum. These seeds treated with Bavistin and Agallal at 0.01 % were found effective in reducing the incidence. Our results are also supported by the results of Hussain et al., (1981) and Hayat (1990) who found that Benlate was more effective against Fusarim oxysporum.

Buchenauer (1978) work on the effectiveness of some new theureido derivatives of systemic fungicides. He concluded that F. oxysporum was significantly controlled by Benomyl. Similar results have been indicated by Kapoor and Kumar (1991), Dwivedi and Pathak (1981), Striber (1985), Mitchell (1987), Sarhan and Kiraly (1981), Shamsher et al. (1983) and Shah Shrivastava (1984). In the present studies, F. moniliforme was the best controlled by Benlate followed by Dithane M-45 the mycelial growth of F. moniliforme was inhibited 72% by Benlate. In case of F. moniliforme Topsin M did not inhibit the mycelial growth significantly. Instead of Topsin M, Dithane M-45 proved to be very effective after Benlate against the *F. moniliforme*. Adachi and Fujita (1986) reported the sensitivities of F. moniliforme to Benzimidazoles. It was noticed that Benlate gave the better results.

Dumitras (1981) reported that F. moniliforme which caused seedling diseases of cotton effectively control by the combination of Topsin M, and Dithane M-45. But in the finding of present studies, Benlate and Dithane M-45 gave good results because Dumitras (1981) did not include Benlate in his experiments. These results are in conformity with the results of Hussain et al. (1988), who found that

Benlate gave good control of F. moniliforme. The Benlate was followed by Dithane M-45 which at 30µg/ml inhibited the mycelial growth of

F. moniliforme. Hayat (1990) also concluded that Benlate was the best to check the growth of F. moniliforme and found Vitavax least significant against the test fungus. Maheshwari et al. (1987) used Benomyl in field trials against F. solani and F. oxysporum which reduced plant mortality and increased yield. Koller et al. (1982) used Benomyl at low concentration to prevent the penetration of F. solani in to host. Lee (1984) found that different seeds dressing fungicides on the germination of soybean seeds and found that Benlate was effective against F. solani which are conformity of our results.

Conclusion

Cultivar NIAB-78 is highly resistant against all fungi isolated and identified while cultivars CIM-443 was highly susceptible due to the presence of maximum number fungi on it. Blotter paper method detected a maximum number of fungi, easy to process in few steps and less time and proved less costly as compare to agar plate method. Seed dressing with available proper fungicides can be recommended to grow susceptible seed. Topspin M is effective for seed treatment.

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