



## Cotton seed borne disease in Golestan Province in Northern Iran

M. ARABSALMANI<sup>1</sup>

<sup>1</sup>Assistant Professor of Agriculture and Natural Resources Research Center of Tehran, Box.3371616738,Ghodoosi St. Varamin, Tehran

Received: 01.02.2015; Accepted: 05.05.2015

**Abstract.** In order to effect of seed mycoflora to incidence of seedling diseases and verticillium wilt, five thousands of cotton seed were collected from five categories (nucleus, super elite, elite, maternal and certified seed) of Sahel cultivar. Seed were delinted with sulfuric acid and treated by sodium hypochlorite 0.5%. Coat and embryo of seeds separated and placed on Potato Dexterosus Agar and Alcol Agar medium and incubated at 18 – 24<sup>0c</sup>. After 5 – 15 days fungi that grew on were isolated and diagnosed. Sand (less than 2mm) were collected on sea coast and sterilized for 30 minutes at 30<sup>0c</sup> for three times and transfer on flower pot. Fuzzy and delinted seeds and embryo (each 400 number) planted inside them and placed at 17 – 25 <sup>0c</sup> on green house. Percent of seedling diseases and seed rot after 15 days and verticillium wilt after 60 days were counted. The results showed that on 63% of coat of seed, 43% of embryo that cultured on PDA, fungi were grew on. Frequency of species, *Rhizoctonia solani*, *Aspergillus flavus*, *Alternaria macrospora* & *A.alternata* and *Fusarium* spp. , on coat seeds were 13.3, 0.25 and 21.4 and on embryo of seeds were 0.4, 32.14 and 33.3 respectively. Percent of healthy seedling, diseased seedling and seed rot that planted on sterilized sand in delinted seeds were 75.5, 3.76 and 12, in fuzzy seeds 67.25, 18.25 and 14.5 and in embryo 74.75, 2.75 and 20.5 respectively. *Aspergillus flavus* (6.6%), *Alternaria macrospora* & *A.alternata* (66.6%) and *Fusarium* spp. (26.6%) were isolated and diagnosed on diseased seedling and rotted seed. *Verticillium dahliae* the casual agent of wilt have not on fungi that isolated and symptom of wilt was not found on plant that planted on flower pot after 60 days.

**Keywords:** Cotton, seed borne, seed, micoflora and seedling disease.

### 1. INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is an fiber plant that presently planted in Golestan Province in Northern Iran. Seedling disease and verticillium wilt of cotton are important diseases in Golestan province (Arabsalmani, 2012). More than 40 fungi have been isolated from diseased cotton seedling, although only some of these have been shown to be seed mycoflora. The fungi involved contaminate the seed prior to harvest (Hillocks, 1992). A number of soil borne and seed borne fungi can infect cotton seedling individually or association as disease complex (Hillocks and Waller, 1997). A wide range of fungi may participate in the process of seed deterioration but a few of these fungi causes pre and post emergence damping off in seedlings. Seed deterioration refers to the breakdown of the cotyledon and embryo tissue within the seed (Kirkpatrick and Rotrock, 2001). Seed borne fungi may effect on uniform emergence, vigorous and uniform stand of healthy seedling. If the fungi have been virulence in seedling, germination can be delayed or may not occur (Arndt, 1953; Lima *et al.*, 1988; Roncadori *et al.*, 1971 and Smith, 1950). In Iran the surveys from 1995 to 2011 for recognize and distribution of the causal agent of diseases on cotton were done, showed that : species of fungi , includes *Alternaria alternata*, *Aspergillus niger*, *Fusarium accuminatum*, *F. solani*, *Pythium ultimum*, *Rhizopus arrhizus*, *Aspergillus* spp., *Rhizoctonia solani*, *Penicillium* spp. are the causal agent of seed decay and pre emergence damping off, and *F. solani*, *F. buharicum*, *F. equiseti*, *F. proliferatum*, *A.alternata*, *Sclerotium rolfsii*, *Pythium ultimum*, *Rhizoctonia solani* are the causal agent of post emergence damping off. Fungi *V.dahliae*, *V.albo-atrum*, *F.oxysporum*

\*Corresponding author. Email address: Omidgh68@gmail.com

f.sp.vasinfestum are the causal agent of wilt on cotton and *F. solani*, *F.semitectum*, *F.proliferatum*, *Fusarium accuminatum*, *S.rolfsii* are the causal agent of root rot. Species *A. alternata*, *A.macrospora*, *Ascochyta gossypina*, *Rhizopus* spp., *Penicillium* spp., *R. solani*, *Bipolaris spicifera*, *Nigrospora* sp., *F. roseum*, *Macrophomina phaseolina* are the causal agent of leaf spot and boll rot. Bacterial blight (incited by *Xanthomonas smithii* subsp. *smithii*) locally and restricted in 2001 to 2004 was incidence in Golestan and Ghorasan – e- shomalii (Arabsalmani, 2012; Mansoori, 1993; Soleimani *et al.*, 1993a and Soleimani *et al.*, 1993b). Verticillium wilt incited by *Verticillium dahliae* Kleb., is the major disease of American upland cotton (*G. hirsutum*) grown in Mazandran, Golestan, Fars and Ardebil Provinces (Arabsalmani, *et al.*, 2011). The current losses of yield in the world are about 1.5 million bales. Estimates of the overall yearly loss in Iran from 1999 to 2004 were recorded 14.23%, 13.67%, 11.73%, 10.65% and 10.42% respectively (Arabsalmani, *et al.*, 2004). Evidence for the seed transmission of *V. dahliae* has been reviewed by Sackston (1982). Microsclerotia are deposited on surface of fuzzy seed during harvest and ginning and a few instances internal infestation has been found (Evans, *et al.*, 1966). In the present study, investigation of effect of cotton seed mycoflora on incidence of seedling disease and verticillium wilt were evaluate.

## 2. MATERIALS AND METHODS

Five thousands of cotton seed were obtained from of the different categories (nucleus, super elite, elite, maternal and certified seed) of Sahel cultivar. They were placed in small cotton bags (25-30cm) and stored in a cool at 4<sup>0c</sup>. Then we have the different methods as bellow (I.S.T.A, 1979).

**A:** Seed were delinted with sulfuric acid and treated in a solution of sodium hypochlorite 0.5% for five minutes prior planting. Coat and embryo of seeds separated. The coat, embryo, delinted seeds and fuzzy seeds were placed in plastic dishes (9 cm diameter) containing of two different media: Potato Dexterosus Agar for isolation of general fungi and Alcol Agar medium for isolation of *V. dahliae*. Dishes were incubated at 18 – 24<sup>0c</sup> under alternate light and darkness for 5 – 15 days (figure 2). After 5 – 15 days fungi that grew on were isolated and diagnosed based on their characteristics and conidial morphology observed at 10 to 60 with a stereomicroscope (Ausher, *etal.*, 1975; Singleton *et al.*, 1992; Agarwal & Sinclair 1997 and Maude, 1998).

**B:** Sand (less than 2mm) were collected on sea coast and sterilized for 30 minutes at 30<sup>0c</sup> for three times and transfer on flower pot. Fuzzy and delinted seeds and embryo (each 400 number) planted inside them and placed at 17 – 25<sup>0c</sup> on green house (figure 3). Percent of seedling diseases and seed rot after 15 days and verticillium wilt after 60 days were counted and pathogens isolated and diagnosed.

## 3. RESULTS AND DISCUSSION

The 20 different fungi isolated were found in coat and embryo that association with the seeds. The most frequent and numerous fungi isolated were either saprophytes or facultative parasites (table 1). The most important being: *Aspergillus flavus*, *Rhizopus* sp. and *Penicillium* spp. The symptom of seedling diseases may be divided for convenience into four groups depending on the stage of development when damage occurs and which part of the plant is affected (Hillocks, 1992). 1) The first stage where damage can occur is immediately after

## Design & modeling a novel Atomic Force Microscope (AFM) for detect roughness in turning machining

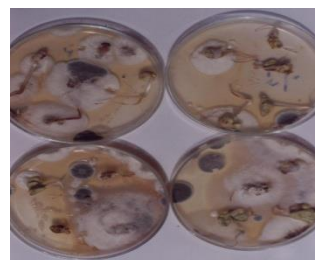
planting, resulting in seed decay. The fungi most often associated with deteriorated seed are *Fusarium* spp. and *Rhizopus* sp ..2) Under sub optimal conditions for plant growth, the newly emerging roots and shoot are vulnerable to attack, resulting in pre emergence damping off. The fungi most often associated with pre emergence damping off are *Fusarium* spp .(figure1),*Pythium* spp. and *Rhizoctonia solani*. 3) Infection of the hypocotyls of seedling after emergence may be occur, and named post emergence damping off. The fungi most often associated with this stage is *Rhizoctonia solani*. 4)Symptoms may become apparent on the cotyledons and first leaves, as a distinct spotting or more generalized blight. The fungi most often associated with this stage are *Alternaria alternata* and *A. macrospora*(figure1).The results showed that on 63% of coat of seed,43% of embryo that placed on PDA, fungi were grew on. Frequency of species, *Rhizoctonia solani*, *Aspergillus flavus*, *Alternaria macrospora* &*A.alternata* and *Fusarium* spp. , on coat seeds were 13.3,0,25 andv21.4 and on embryo of seeds were 0,4,32.14 and 33.3 respectively(table4).

Percent of healthy seedling, diseased seedling and seed rot that planted on sterilized sand in delinted seeds were 75.5, 3.76 and 12, in fuzzy seeds 67.25,18.25 and 14.5 and in embryo 74.75,2.75 and 20.5 respectively(table2). *Aspergillus flavus*(6.6%),*Alternaria macrospora* &*A.alternata*(66.6%) and *Fusarium* spp.(26.6%) were isolated and diagnosed on diseased seedling and rotted seed(table3) . *Verticillium dahliae* the casual agent of wilt have not on fungi that isolated and symptom of wilt was not found on plant that planted on flower pot after 60 days.

Thus, if seeds were delinted and treatment with suitable fungicides, amount of 15% of seedling diseases associated to contamination of seeds may be controlled in Golestan provience. Acid delinting of seed and treatment with suitable fungicides prevent seed transmission(Maude,1998).Acid delinting of seed prior to storage will remove seed coat fuzz and adhering trash, which might harbor microbial contaminants. Low temperature germination is a good indicator of field performance. Seeds which germinate at 180c are usually free of infection and are also more able to with stand attack by soil microorganisms.



**Figure 1.** Colony of *Fusarium* and *Alternaria* grow on PDA.



**Figure 2.** Colony of Fungi grow on coat and embryo on PDA..



**Figure 3.** Planted of seeds on flower pot and presents of seedling disease.

**Table 1.** Frequency of fungi isolated from coat and embryo of Sahel cultivar.

fungi	coat	embryo	frequency
<i>Alternaria alternata</i>	-	+	32.14
<i>A. macrospora</i>			
<i>Fusarium</i> spp .	-	+	33.3
<i>Alternaria alternata</i>	+	-	25
<i>A. macrospora</i>			
<i>Fusarium</i> spp .	+	-	21.4
<i>Aspergillus flavus</i>	-	+	4.4
<i>Rhizopus</i> sp .	+	-	18.6
<i>Rhizoctonia solani</i>	+	-	13.3
<i>Penicillium</i> spp .	+	-	20
<i>Nigrospora oryzae</i>	+	-	10.7
<i>Curvularia</i> sp .	+	-	2.1
<i>Cladosporium</i> sp .	+	-	20.1
<i>Trichoderma</i> sp .	+	-	3
<i>Ulocladium</i> sp .	+	-	2
<i>Chaetomium</i> sp .	+	-	1.5
<i>Cephalosporium</i> sp .	+	-	2.5
<i>Bipolaris</i> sp .	+	-	1.5
<i>Stril mycellium</i>	+	-	2
Other fungi	+	-	3.5

**Table 2:** Percent of diseased and healthy seedling on flower pot.

Seed decay	Diseased seedling	healthy seedling	Type of seed
14.5	18.25	67.25	Fuzzy seed
12	3.75	75.5	Delinted seed
20.5	2.75	74.75	Embryo of seed

## Design & modeling a novel Atomic Force Microscope (AFM) for detect roughness in turning machining

**Table 3.** Frequency of fungi isolated from coat and embryo on flower pot.

<i>Alternaria alternata</i> <i>A. macrospora</i>	<i>Fusarium spp.</i>	<i>Aspergillus flavus</i>	Other fungi
66.6	26.6	6.6	0.2

**Table 4.** Frequency of fungi isolated from coat and embryo on PDA.

	<i>Rhizoctonia</i>	<i>Aspergillus flavus</i>	<i>Alternaria alternata</i> <i>A. macrospora</i>	<i>Fusarium spp.</i>
Embryo	0	4	32.14	33.3
Coat	13.3	0	25	21.4

### Acknowledgment

I thank the Cotton Research Institute of Iran for supporting this study.

### REFERENCES

- [1] Agarwal, V.K and J . B . Sinclair.(1997). "principles of seed pathology" . CRC Press. 539 p.
- [2] Arndt, C. H. 1953."Survival of colletotrichum gossypii on cotton seed in storage". Phatopathology 43: 22.
- [3] Arabsalmani, M.2012.Importance and distribution of causal agent of cotton diseases in Iran.The1st international conference on science,industry and trade of cotton.Oct.2-4,2012 ,Gorgan,Iran.
- [4] Arabsalmani,M.,Okhovvat,S.M.,Sharifitherani,A.,Nikkha,M.j. and Safaie,N.2011.Epidemiology of Verticillium wilt of cotton in golestan provience:effect of Verticillium wilt on quantitative and qualitative characters of cotton . Iranian Journal of Plant pathology47(1):1- 18.
- [5] Arabsalmani, M. Rahnama, K., Rahimian, H. and Banihashemi, Z. 2004. Evaluation of percent of losses due to Verticillium wilt in cotton in Golestan Province. Iranian Plant Protection Congress, 28 Aug. – 1 Sept. 2004.
- [6] Ausher, R., Katan, J. and Ovadia, S. (1975). "An improved selective medium for the isolation of Verticillium dahliae". Phytoparasitica 3: 193-137.
- [7] Evans, G ., Wilhelm . S . and W . C .Snyder. 1966. "Dissemination of the Verticillium wilts fungus". Plant Dis. Rep. 44. 901.
- [8] Hillocks, R. J. 1992. "Cotton Diseases". CAB. International, Wallingford, UK. 415.
- [9] Hillocks, R . J . and J.M . Waller. 1997. "Soil borne Diseases of Tropical crops" . C . A . B . Press.
- [10] I.A.T.A.(International Seed Testing Association).1976.International rules for seed testing .SEED Scin. and Tecknol.4:8-49.
- [11] Lima , E . F , Carvacho , J . M . C . and L .P . Carvalh . 1988 . "Survival of colletotrichum gossypii var . Cephalosporioides on cotton ( Gossypium hirsutum L . ) seed" . Fitopatol . Brasileria 13 : 247 .

- [12] Mansoori , B . 1993 . "Seed – borne fungi of cotton in Iran". Proceedings of the II the plant protection Congress of Iran". 115.
- [13] Roncadori , R . W., Mc carter, S. M . and J . L .Craford . 1971 . "Influence of fungi on cotton seed deterioration prior to harvest ". Phytopathology . 61 . 1326.
- [14] Soleimani , M . J . , GH. A .Hedjaroude. and J . ZAD . 1993a." Studies on Pathogenicity of some seed – borne Fusarium species on cotton seedling. Iran". J. Plant path. 29: 35 – 41.
- [15] Solemani , M . J . , GH. Hedjaroude . A . and J . ZAD . 1993b. "Survey on mycoflora of cotton seed in Iran" . J . Plant . 29 : 132-139 .
- [16] Smith, A . L .1950. "Ascochyta seedling blight of cotton in Alabama in 1950 ". Plant Dis. Rep . 34 ; 233 .
- [17] Sackston,W.E.1983.Epidemiology and control of seed borne Verticillium spp.causing vascular wilt.Seed science and technology11,731-47.
- [18] Singleton, L. L., Miral, J. D. and Rush, C. M. 1992. Methods for Research on Soilborn phytopathogenic Fungi.American Phytopathological Society, St. Minnesota, 265p.
- [19] Kirkpatrick, T. L. and Rotrock, C. S. 2001." Compendium of Cotton Disease".APS Press 77 p.
- [20] Maude, R.B.1998.Seeborne Diseases and Their Control.CAB Press478pp.