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Impact of storage period and temperature on the pathogenic behaviour of *Fusarium solani* on cotton *(Gossypium hirsutum* L.) seeds

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Abstract

Fusarium solani grows well at temperature of 25 ºC than at 22 ºC on potato dextrose agar medium and covered the 9 CM petri plate in 192 hours. While the best temperature for its growth was found to be 20 ºC in storage conditions. *Fusarium* specie, *solani* and *semitectum* (*Pallidoroseum*) showed increasing trend in their growth during two years span in storage. But the other fungi isolated were *Aspergillus* sp, *Rhizopus* sp., *F. solani , Macrophomina phaseolina, Arthrobotrys* spp , *Chaetomium* sp and *F. semitectum* (*Pallidoroseum*) have 1800, 2812, 200, 100, 100, 100 and 500 times spread, respectively in two years growth period in storage conditions at 25 ºC. The preponderant trend of the isolated fungi were indicating their degree of aggressiveness. The pathogen *F. solani* caused 100% mortality in all the treatments and reduced germination greatly in fuzzy and acid delinting practices. Cotton variety FH-901 showed resistance both in pots and fields against the pathogenic fungus *F. solani.* This variety gives 78 and 85% germination in pots and in fields respectively as compared with other three (FH-207, FH-114 and NIAB-78) varieties. 100% recovery of the pathogenic fungus *F. solani* was recorded from dried seedlings when incubated for isolation both in PDA medium and standard blotter test. The pathogen *F. solani* grows well at temperature of 25 ºC than the temperature of 22 ºC on synthetic potato dextrose agar medium.

Keywords: Cotton (*Gossypium hirsutum* L.), *F. solani*, root rot, storage period, seed germination, temperature.

Introduction

Cotton, *Gossypium hirsutum* L. is the most important crop of the world Amarica, Africa, South Asia including Pakistan. Pakistan economy has agro-base infra Structure. Agricultural commodities contributes more than 25% share in the GDP of the economy and cotton is the main cash crop of the country. Any effort to increase the cotton production will help to boost up the economy of Pakistan. Diseases like, *Fusarium* wilt, seedling blight/boll rot, *Verticillium* wilt, Black arm/Angular leaf spot and CLCuV are more common in the World and causes 12% an average yield reduction in cotton (Idrees *et al*., 2000 and Neergaard, 1979). The pathogenic fungus *F. solani* also caused hypocotyls rot and root rot disease (Colyer, 1988; King and Presley, 1942). The most common fungi associated with cotton diseases are *Fusarium* spp., *Colletotrichum gossippi*, *Rhizopus* spp. (Roy and Bourland, 1982; Johnson *et al.*, 1983; Mauk and Hine, 1988, Hillocks, 1992). *Verticillium* wilt in cotton has been reported in southern Spain (Bejarano-Alcazar *et al*., 1997). In recent years cotton have been cultivated on an area of 3192.6 thousand hectares yielding 679.17 kg. seed cotton per hectare, i.e., approximately 7.55 mounds per acre (MINFAL, 2006) which is very low. This reduction in yield is mainly due to the use of morbid seed and prevalence of diseases in the country. Therefore, it is contemplated to undertake studies on the wilt problem. Studies have been carried out on the effect of Temperature and Storage period to see the effect of the disease on seed in storage and on seedling and crop in field conditions.

Materials and Methods

Effect of different storage periods and temperatures on cotton seed were studied in laboratory at 6, 12, 18 and 24 months storage period and 0, 10, 20, 30 and 40 ºC temperatures. These studies were carried out *in vivo* and *in vitro* conditions. The cottonseed samples obtained were wrapped in paper envelops and labeled them properly by observing ISTA rules. Samples were kept at above mentioned temperature range for the prescribed period to examine the spread of the *F. solani* alongwith other fungal seed borne infection. Surface sterilized seeds were studied with an international accepted procedure for pretreatment,

is soaking for 5 minutes in 1.0% sodium hypochlorite and plating immediately without washing the treated seeds (Thomus, 1985).

The soil samples were studied by using the filter method and dilution plate method for identification of fungus (Dasgupta, 1968). These collected samples have been studied one by one at different temperature ranges and storage periods. The samples were kept in the laboratory at 25±1 ºC and tested for fungal infection spectrum by using the standard blotter method (ISTA 1980). After seven days of incubation in dishes these were examined under stereomicroscope for the growth of pathogenic fungus *F. solani* in aggravation of other fungi. Each seed in the dish was observed and fungi recorded were marked after identification directly on visual growth pattern basis (Nelson *et al*., 1983). Those organisms that have not been identified were water mounted on slides and observed for identification under microscope (Ellis 1971 and 1976; Booth, 1971). The culture of *F. solani* was also identified and purified on PDA medium. These dishes of purified culture were kept in incubator at 22 ºC temperature.

The old culture was revived along with the fresh one on synthetic nutrient medium in dishes to test its viability. Inoculated dishes were kept in the incubator at 22±1 ºC temperature in the laboratory and in laminar flow having temperature thermometer attached with in range of maxima 0 - 50 degree and range of minima 0 - 30 degree temperature marked on it. The growth of the fungus was recorded after scheduled time on the medium. For inoculation 1cc distilled water injected in preserved dry cultured test tubes to prepared inoculation solution. These tubes were kept in laboratory for 7 days period and then inoculated in fresh PDA dishes. The radial growth of the fungus was measured to assess its physiological characters. Percentage increase in the spread of infection in fungi at storage conditions have been calculated by the formula developed as given below.

$$\%\text{Increase of infection} = \frac{\text{Maximum Inf. \%} \ (-)\ \text{Minimum Inf. \%}}{\text{Minimum Inf. \%}} \times 100$$

Results and Discussion

Identification of *F. solani*

i. Physiological studies

For inoculation 1cc distilled water have been injected in preserved dry cultured test tubes to prepared inoculation solution. These tubes were kept in laboratory for 7 days period and then inoculated in fresh PDA dishes. The radial growth of the fungus was measured (Table.1). It is clear from the table data that the pathogen *F. solani* grows well at temperature of 25 ºC than the temperature of 22 ºC on synthetic potato dextrose agar medium. This culture has been maintained for uses in further studies.

Table. 1: Mean radial growth of *f. Solani* on pda medium.

Hours	Mean fungal growth in cm	
	Incubator 22 ºC	Laboratory laminar flow 25 ºC
24	START	START
48	0.3	0.5
72	2.2	2.5
96	3.5	4.7
120	3.9	6.0
144	4.7	6.7
168	5.5	7.9
192	7.75	9.0

ii - effect of storage period and temperature on seed germination

Storage period and temperature, both effect the seed germination in invitro conditions at different temperature ranges and storage periods starting from 0 days to 24 months i.e. two years period of span (Table. 2).

Table. 2: Effect of storage period and temperature on cotton seed mycoflora collected from Bahawal-pur region.

Fungi	Storage period in months with infection percentage as recorded at 25 °C				
	0	6	12	18	24
Aspergillus sp	2.0	22.0	38.0	40.0	38.0
Rhizopus sp	1.7	19.0	28.0	32.0	41.0
F. solani	1.0	2.0	3.0	2.5	3.0
Macrophomina phaseolina	3.0	10.0	8.0	32.0	0.0
Arthrobotrys sp	2.0	0.0	0.0	1.0	0.0
Chaetomium sp	4.0	1.0	0.0	0.0	0.0
F. semitectum	1.0	4.0	4.0	3.0	6.0

The table data clearly shows that the fungi belonging to the genous *Aspergillus* sp, *Rhizopus* sp, and *Fusarium* sp, increases gradually in the storage period of about 6, 12, and 18 months but *Macrophomina phaseolina* drastically checked and become zero in 24 months storage period at room temperature of 25 ºC (Table. 2). The room temperature maintained by instilling temperature

maintaining units and thermo-stated fitted to control it through out the period. The preponderant trend of these fungi indicates their degree of aggressiveness. All these fungi produced vivo-toxins that effect the host in one way and the other (Neergaard, 1979). The fungal spores, fragments and propagules are caused deleterious effects on the plant growth and seed vigour. *Aspergillus* sp. and *Rhizopus* sp. are common storage fungi. These fungi spreads during suitable storage conditions and contaminate the storage commodities, like cotton seed, cotton seed cake, etc. *Macrophomina phaseolina* looses its viability after 18 months in the storage. *Arthrobotrys* sp and *Chaetomium* sp both restricted to their growth after a half year and a year respectively. Tanaka (1994) in Brazil studied the effect of temperature and storage period in co-relation with relative humidity on anthracnose disease in cotton and reported that 8 month storage of seeds at 14 ºC and 90% RH produced germination and emergence superior than other two conditions.

F. solani and *F. semitectum* showed increasing trend in their growth during two years span. All these fungi i.e. *Aspergillus* sp., *Rhizopus* sp., *Fusarium solani*, *Macrophomina phaseolina*, *Arthrobotrys* sp., *Chaetomium* sp. and *F. semitectum* have 1800, 2812, 200, 100, 100, 100 and 500 times spread in two years growth period in storage (Table. 3). These fungi were identified by (Booth 1971; Dasgupta, 1968; Neergaard, 1979; Nelson *et al*., 1983).

Table. 3: Percentage increase in fungi during 24 months period.

Fungi	Percentage infection spread in two years
Aspergillus sp	1800
Rhizopus sp	2812
F. solani	200
Macrophomina phaseolina	100
Arthrobotrys sp	100
Chaetomium sp	100
F. semitectum	500

The pathogenic fungus *F. solani* under consideration increases gradually during 6, 12, 18 and 24 months period by 50, 200, 200 and 200 times respectively (Table. 4). Fig. 1 showed the macroconidia of pathogen with three septations. Microconidia of the pathogen was not seen and found in this strain of *F. solani* fungus.

Table 4: Periodical spread of *Fusarium solani* in storage.

Storage period in (Months)	Pathogen spread in times (%)
6	50
12	200
18	200
24	200

Pathogenicity tests

Table 5: *In Vivo* Pathogenicity tests in cotton seeds against *f. Solani*, the wilt causing fungus.

Sr. No.	Patho-gen	Mean *G %		Mean **M %	
		Fuzzy	Deli-nted	Fuz-zy	Delin-ted
1	*F.solani*	55.73	36.76	100	100
2	Healthy	93.33	80.00	0.00	0.00

*****Germination *Mortality

In pathogenicity test studies delinting practices could not showed any pronounced effect on cotton seed germination when seeds sown in pots amended with culture of *F. solani* and non amended pots filled with sterilized soils. These showed 93.33 and 80% germination in fuzzy and delinted treatments in control studies respectively. The pathogen caused 100% mortality in all the treatments with the culture of pathogenic fungus *(F. solani)* in fuzzy and delinted treatments in pathogenicity test studies where culture of pathogenic fungus *(F. solani*) applied in soil in pots (Table. 5).

Isolation from soil

The pathogenic *F. solani* also isolated from soil samples in 100% frequency collected from infected cotton fields.

Table. 6: Effect of temperature on the fungal growth and seed rot development.

Growth of *F. solani* (CM)	Storage Temp. °C	Seed rot in dishes (%)
0.0	0	0
2.0	10	0
9.0	20	95
8.5	30	83
4.0	40	10

The best temperature for the growth of *F. solani* is 20 ºC on seeds in storage while reduction in fungal growth was started at 30 ºC and decreased to less than half of it at temperature of 40 ºC (Table. 6). The growth of pathogenic fungus *F. solani* was very slow at temperature of 10 ºC. This pathogenic fungus grows up to 0.0, 2.0, 9.0, 8.5 and 4.0 centimeters at temperatures of 0.0, 10.0, 20.0, 30.0

and 40 ºC respectively (Table. 6). The best minimum temperature for the fungal growth on PDA is 20.0 ºC in storage, where it grows up to maximum level of 90 mm in pyrex pettri plates. These studies provide information about the maximum requirement of temperature for fungus growth. The 95 and 83 percent seed rot occurs at temperatures of 20.0 and 30.0 ºC where fungal growth was recorded as 9.0 and 8.5 centimeters. Such type of syndrome was not recorded at 0 ºC. Zhang *et al.* (1996) studied the 97 *Fusarium* specie out of which 11:3 were the *F. solani* and *F. oxysporum*. These reduced seedling growth and caused necrotic lesions on taproots and secondary roots. They reported that *Fusarium solani* isolates were more virulent on cotton seedlings than those of *oxysporum.* In pathogenicity of 4 months old seedlings and on 2 months old cuttings in green house against *F. solani* with (1 × 107 conidia/ml) at 25 ºC for 7–10 days. All inoculated plants developed wilt symptoms in the field within 2 weeks after inoculation on commercial field lavender in China (Ren *et al*., 2007).

Four cotton varieties, FH-207, FH-114, FH-901 and NIAB-78 were studied in the laboratory and in field. Each variety exhibit the different behaviour in each medium. But FH-901 showed resistance in pots and in fields against the pathogenic fungus *F. solani.* This variety gives 78 and 85% germination in pots and in fields respectively. It only showed 6% seed rot and no seedling mortality was recorded. The pathogenic fungus *F. solani* was not recorded in pots and fields on variety FH-901. But the other three varieties FH-207, FH-114 and NIAB-78 showed 4.5, 3.5 and 20 percent seedling mortality in field at temperature of 41 ºC in average and spots on bolls (Fig. 2) according to meteorological data recorded. 100% recovery of the pathogenic fungus *F. solani* was recorded from dried seedlings (Table. 7 & Fig. 1). Different temperature and storage periods greatly effects longevity of the fungus *F. solani*. It should be necessary to assess the disease prevallence occasionally in cotton growing areas to restrict spread of the disease in disease free localities to enhance crop yield.

Table. 7*:* Effect of temperature on seed germination.

Varieties	Temperature ºC		% seed rot syndrome	G % age		% Seedling mortality		% isolation of *F. solani*	
	Lab.	Field	Lab.	Pot	Field	Pot	Field	Pot	Field
FH-207	20	41*	46	61	48	4.5	9.5	100	100
FH-114	20	41*	49	46	53	3.5	7.5	100	100
FH-901	20	41*	6	78	85	0.0	0.0	0.0	0.0
NIAB-78	20	41*	55	40	45	20	18	100	100

* Meteorological data report

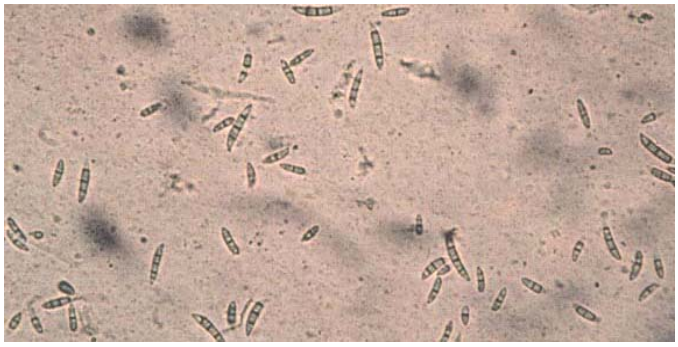

Fig. 1: *Conidia* of (*F*. solani) isolated from diseased cotton plant.

Fig. 2: Mature diseased boll with spots.

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