STUDY ON MYCOFLORA ASSOCIATED WITH SEEDS OF DIFFERENT CITRUS SPECIES

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Abstract

This study was carried out on the mycoflora associated with seeds of different citrus species. Citrus seed material was collected from districts of Punjab, i.e. Multan, Sargodha and Khanpur. Standard methods were applied for the isolation and identification of fungi. A total of 11 fungi including Aspergillus fumigatus, Aspergillus flavus, Dreschslera tetramera, Alternaria alternata, Curvularia lunata, Macrophomina phaseolina, Aspergillus niger, Fusarium solani, Fusarium moniliforme, Rhizopus and Penicillium spp were isolated from the seeds of citrus. For control of isolated seed-born fungi, 3 recommended fungicides such as Ridomil Gold, Bavistin, Score and two chemical Salicylic acid and Boric acid, were used at 20, 30, 40 mg/10 mL and 5, 6, 7 µL/10 mL, respectively and chemical with 20, 30, 40 mg/10 mL. All these fungicide and chemicals significantly reuced with population of all fungi present in naturally infected seed samples. Ridomil Gold and Salicylic acid were found to be the best for the control of seed-born fungi of citrus seed at 40 mg/10 mL. The isolation and identification of different mycotoxins is essential to study health status of the citrus consumers and to safeguard the standards of WTO.

1.INTRODUCTION

According to production, Citrus are main fruit crops among tropical and subtropical parts worldwide. About 106 MMT citrus fruit is produced worldwide. In Pakistan, among fruit crops, citrus lies number one position in area (192 thousand hectare) and production (2.5 MMT) as well (Khan et al. 2014). Oranges account for 60% in whole production (Oreopoulou & Tzia, 2007). Genus *Citrus* includes mandarin (*Citrus reticulata*), orange (*C. sinensis*), pomelo (*C. maxima*), kumquat (*Fortunella margarita*), lemon (*C. limon*), lime (*C. aurantifolia*), and some hybrids of mandarin × pomelo or mandarin × orange (Wang, 2012).

Several kinds of moulds are distributed all around in our nature. Mould spores can be easily found even at high altitudes. These spores can be dispersed via wind and air currents. They can be spread by insects, rodents, and mammals. The moulds are involved in metabolic activities i.e. decomposition of organic substrate and recycling of organic molecules. Mycotoxins are moulds which are toxic metabolites. These metabolites are produced by filamentous fungi present in contaminated food commodities (Jeff-Agboola et al. 2012). The postharvest fungi tend to produce mycotoxins under high relative humidity and adequate temperature (Shenasi et al., 2002).

It was reported several factors are involved in fungal deterioration of stored seeds, but among these factors insects, initial inoculum load, water activity and storage temperature are found to be most important (Passone et al., 2009).

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It was reported that when fruit juices are contaminated by *Aspergillus, Penicillium, Fusarium, Byssochlamys, Neosartorya,* these moulds become a part of entire food chain (Corbo et al, 2010). *Aspergillus fumigatus* is causative agent of Aspergillus disease in humans and other livestock (Arrus et al., 2005). Aspergillus disease includes allergic syndromes, aspergilloma and chronic or acute invasive aspergillosis (Snelders et al. 2012). Aflatoxins are metabolites produced by *Aspergillus flavus* and *A. parasiticus* (Williams et al., 2004). Aflatoxins are carcinogenic, teratogenic, hepatotoxic, mutagenic, and immunosuppressive, and can inhibit metabolic systems (Arrus et al., 2005). They are responsible for significant high economic losses in food industries (Novoa et al., 2006).

Gummosis is caused by *Macrophomina phaseolina* (Singh 1996). The gummosis is the most devastating disease of citrus in under dry and rainy period. Weak and injured trees are susceptible to gummosis infection. Several gum exudes are found from gum pockets located on three trunks. The wood beneath gum pockets become pink /orange in color. The root, stem, leaves, blossoms and fruit become week (Das et al. 2010).

Several fungicids are being used to control seed borne diseases from last decade. But nowadays, considerable attention has been given to the use of natural compounds, such as essential oils (EOs) to avoid postharvest decay caused by fungal strains in fruit crops (Xing et al., 2010; Atia, 2011; Kumar et al., 2011). Several research studies are reported on fungi and mycotoxins. But there is a need of accurate identification of microorganisms (Pitt and Hocking 2009).

In this study, Citrus seed was collected from districts of Punjab, i.e. Multan, Sargodha and Khanpur for the isolation and identification of seed borne fungi. Three recommended fungicides such as Ridomil Gold, Bavistin, Score and two chemical Salicylic acid and Boric acid, were used were used to control isolated fungal species. This study will help us to use standard method of fungal isolation and will help us to identify the topmost fungicide for significant control of seed borne fungi.

2. MATERIALS AND METHODS

The studies on mycoflora associated with seeds of different citrus *spp* were conducted in department of Plant Pathology Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi during 2010-2011.

2.1 COLLECTION AND PREPARATION OF SEED SAMPLES

The fields were selected and surveyed at Multan, Sargodha and Khanpur in the Punjab. These places deal with all kind of seeds including fruit crops. The following six citrus cultivars were selected.

Table 2.1: Table showing six citrus cultivars used in this study

Sr. No.	Common Name	Botanical Name	Purpose	Distribution
1.	Kinnow mandarin	*Citrus nibilis*	Commercial	Punjab
2.	Sweet orange	*Citrus sinensis*	Commercial	Punjab
3.	Grape fruit	*Citrus paradisi*	Commercial	Sindh, Punjab
4.	Lemon	*Citrus limon*	Commercial	Sindh, Punjab
5.	Rough lemon	*Citrus jambhiri*	Rootstock	Punjab
6.	Sour orange	*Citrus aurantium*	Rootstock	KPK

The seed samples were preserved in paper bag and stored at room temperature ($25^{O}C$). For sterilization 5-10 seeds of each spp were drawn and sterilized with 1% sodium hypochlorite (NaOCl) for one minute. Sterilized seed were rinsed three times with distilled water and then dried on blotter paper (Mittal et al. 1999).

2.2 DTECTION OF SEED MYCOFLORA

For quick identification of seed borne mycoflora blotter paper test was most useful (Neergard, 1978 and ISTA, 1993).

2.3 INCUBATION TEST FOR DETECTION OF SEED BORNE FUNGI

Incubation tests are primarily used to determine the kind, amount and distribution of inocula. Major five types of incubation tests are available but in this study two types of tests were performed; agar plate method and second is blotter paper method. The results may reveal that incubation test is better to check the seed borne mycoflora for citrus spp.

2.4 BLOTTER PAPER METHOD

Petri plates were washed with distilled water and then dried in an oven at $100^{O}C$ for 10 minutes. Three layers of blotting paper (8 cm, diameter) were cut according to the size of Petri plate and placed at the bottom and moistened with water. Superfluous water was drained off. Five seeds of each species were surface sterilized with 1% Clorox for 2 minutes, rinsed with sterilized water and placed in each dish. Plates were incubated at $25^{O}C$ for eight days under alternating cycle of 12 hours day and night fluorescent light.

2.5 AGAR PLATE METHOD

For agar plate method, potato dextrose agar (PDA) medium was prepared as under:

Agar-agar	20gm
Potato	250gm
Dextrose	20gm

These ingredients were dissolved in 1000ml distilled water in conical flask and autoclave at $121^{O}C$, 15 psi for 20 min. In each plate of 9cm diameter, 20ml of melted sterilized medium was pour and solidified at room temperature under a laminar flow cabinet (Galair SN 8201, Italy). Five surface sterilized seeds after washing were placed in each dish replicated four times. The plates were incubated at $25^{O}C \pm 2$ for seven days with alternate light and dark 12 hours cycle. Colonies of fungi and fungal species were examined regularly.

2.6 IDENTIFICATION OF FUNGI

All the petriplates in both the methods were examined under a stereomicroscope (SZH-ILLB, 604131 Olympus Japan) and the number of seeds infected and the fungal colonies developed were calculated as under:-

$$\%\ Frequency = \frac{No.of\ seeds\ infested}{Total\ No.of\ seed\ Plated} * 100$$

In order to properly identify the fungi, microscope glass slides were prepared. Small material of fungal hyphae, mycelia or spores were taken with a sterilized needle, stained with Lectophenol and observed under a compound microscope (BHZ 105411 Olympus Japan) at 40-100X magnification. The fungi were identified on the bases of their typical structures and basic characters as suggested by Barnett (1960) and Melone and Masket (1964). The frequency of each fungus was determined in the percentage from the colonies of all fungi developed.

2.7 EFFECT ON GERMINATION

A total of 15 seed samples, naturally infected with seed borne fungi, were tested through germination. One hundred seeds fo each samples were placed separately on anchor brand paper (24x48cm) in four rolls, each roll with 50 seeds. Papers were put in polyethylene bags and incubated at $25^{O}C \pm 2$ for 12 – 15 days. Water was added to keep the paper moist. In one set of experiment, using healthy (pathogen free) seeds were considered as control and no pre-treatment was given to any seed sample in case of control. After 12 days, the rolled paper was exposed and seedlings were examined individually for three categories: normal seedling, ungerminated seeds and rotted seeds.

The fungi were examined under stereo microscope on germinated and ungerminated seeds basis. Diseased portion of seedlings was cut and plated on PDA to confirm these pathogens. Data regarding germination was recoded 16 days after placing and the result was recorded in percentage.

$$\%\ Frequency = \frac{No.of\ seeds\ infested}{Total\ No.of\ seed\ Plated} * 100$$

2.8 CHEMICAL SEED TREATMENT

Soaking method followed by Gangopadhyay and Kapoor, (1977) was used with some modifications. Ten gram seeds of citrus were soaked in different concentration of fungicides (Ridomal Gold, Bavistin and Score) and chemicals (salicylic acid and boric acid) and left of one hour to enable the seeds to absorb the fungicides and chemicals. After treatment, seeds were air dried for 30 minutes and analyzed for their efficacy against seed-borne mycoflora by using standard blotter paper method. The details are follows:-

A total of 100 seeds of citrus *spp*, naturally infected with important seed borne fungi were treated individually with the fungicides at the rate of 15, 20, 30mg/10ml and 5, 6, 7µl/10ml, respectively, and chemicals with 15, 20, 30mg/10ml. five seeds were plated in each patri dish. Experiment was conducted in four replications with five seeds each replication and incubation of treated seeds were carried out at $25^{O}C$ for eight days. 100 seeds were also plated on blotter paper without any treatment of fungicide to serve as control. Seeds were examined under stereoscopic microscope and the fungi were identified based on habit characters on seed and colony characters on blotter paper around the seed. Results expressed in percentage.

3. RESULTS AND DISCUSSION

A study was carried out in 2012-2013 to determine the number of fungi associated with seeds of different citrus species and to find out impact of seed brone fungi on seed health followed by treatment with different fungicides.

3.1 ISOLATION OF FUNGI

Seeds of six citrus species were examined for the mycoflora associated with them, and two standard methods of isolation ie.e. blotter and agar plate method were employed for this study. A total of 11 fungi including *A. fumigatus* Fres, *A. flavus* Link ex Gray, *Dreschslera tetramera* (Machinney) Sub and Jam, *A. alternata* Nees, *Curvularia lunata* (Wakker) Boed, *Macrophomina phaseolina* (Tassi) Goid, *A. nigar* van Teighem, *F. solani* (Mart.) App and WR, *F. moniliforme* Sheldon, *Rhizopus and Penicillium* spp were isolated from the seeds of citrus. These fungi were belonging to three families and three orders and eight genera i.e., *Dreschslera, Penicillium, Rhizopus, Macrophomina, Alternaria, Fusarium, Curvularia lunata* and *Aspergillus* (Table 3.1).

Table 3.1: Seed-borne fungi belonging to various orders and families

Fungi	**Family**	**Order**
Aspergillus flavus *Aspergillus niger* *Aspergillus fumigatus* *Penicillium spp.*	Moniliaceae	Moniliales
Alternaria alternate *Rhizopus spp.* *Curvularia lunata* *Dreschslera tetramera*	Dematiaceae	Moniliales
Fusarium solani *Fusarium moniliforme*	Tuberculariaceae	Moniliales

3.2 STUDY ON SEES HELTH TESTING METHODS

In the present study, it was found that higher number of pathogens developed on blotter paper method as compared to agar plate method e.g. Kinnow mandarin *(Citrus nobilis)* from Multan was found infected with the *Aspergillus flavus, Aspergillus niger, Rhizopus* spp., *Pencillium* spp and *Alternaria alternata* with frequencies of 80, 60, 60, 40 and 80% on blotter paper. The number of fungi was observed on PDA such as *Aspergillus flavus, Aspergillus niger, Rhizopus* spp., *Pencillium* spp and *Alternaria alternata* having the 20, 40, 40, 20 and 60 %age. Similarly sweet orange *(Citrus sinensis)* had only four fungi, *Fusarium solani, Aspergillus flavus, Alternaria alternata* and *Fusarium moniloforme* with 60, 40, 60 and 80 %age infection in blotter paper method but the number of fungi observer on PDA such as *Fusarium solani, Aspergillus*

flavus, Alternaria alternata and *Fusarium moniliforme* with the percentage of 60, 20, 20 and 40 %. Grapefruit *(Citrus paradise)* from Multan was found infected with *Dreschslera tetramera, Curvularia lunata Alternaria alternata* and *Pencillium* spp with percentage of 40, 40, 80 and 60% on blotter paper and on PDA with the percentage of 40, 40, 20 and 60%. On lemon *(Citrus limon)* three fungi, *Rhizoctonia solani, Alternaria alternata* and *Aspergillus fumigatus* developed with the percentage of 60, 60 and 40 on blotter paper and 20, 40 and 20 on PDA. Similary rought lemon *(Citrus jambhiri)* was infected with four fungi i.e., *Aspergillus fumigatus, Rhizopus* spp, *Rhizoctonia solani* and *Fusarium moniliforme* with the percentage of 80, 60, 80, 40 and 40, 40, 20, 40 on blotter paper and PDA, respectively. Sour orange *(Cirtus aurantium)* was infected with *Alternaria alternata, Curvularia lunata, Rhizopus* spp, *Rhizoctonia solani* and *Rhizoctonia solan* with the percentage of 40, 40, 60, 80, 60 and 40, 60, 40, 60, 20 on blotter paper and on PDA, respectively (Table 3.2).

Table 3.2: Comparative study of Citrus Seed Health Testing Method (Multan)

Common Name	Fungi Identified	**Multan**			
		Blotter Paper		PDA	
		No. of seed infected	%age infection	No. of seed infected	%age infection
Kinnow mandarin	*Aspergillus flavus*	4	80	1	20
	Aspergillus niger	3	60	2	40
	Rhizopus spp.	3	60	2	40
	Penicillium spp.	2	40	1	20
	Alternaria alternata	4	80	3	60
Sweet orange	*Fusarium solani*	3	60	3	60
	Aspergillus flavus	2	40	1	20
	Alternaria alternata	3	60	1	20
	Fusarium moniliforme	4	80	2	40
Grape fruit	*Dreschslera tetramera*	2	40	2	40
	Curvularia lunata	2	40	2	40
	Aspergillus flavus	4	80	1	20
	Penicillium spp.	3	60	3	60
Lemon	*Rhizoctonia solani*	3	60	1	20
	Aspergillus fumigatus	3	60	2	40
	Alternaria alternata	2	40	1	20
Rough lemon	*Aspergillus fumigatus*	4	80	2	40
	Rhizopus spp.	3	60	2	40
	Rhizoctonia solani	4	80	1	20
	Fusarium moniliforme	2	40	2	40
Sour orange	*Alternaria alternata*	2	40	2	40
	Curvularia lunata	2	40	3	60
	Rhizopus spp.	3	60	2	40
	Rhizoctonia solani	4	80	3	60
	Rhizoctonia solan	3	60	1	20

In Sargodha Kinnow mandarin *(Citrus nobilis)* was found infected with *Aspergillus flavus, Aspergillus niger, Rhizopus* spp., *Pencillium* spp and *Alternaria aternata* with percentage of 60, 80, 60, 40, 20 and 40, 20, 40, 40, 20 on blotter paper and PDA, respectively. Similary sweet orange *(Citrus sinensis)* had only four fungi, *Fusarium solani, Aspergillus flavus, Alternaria alternata* and *Fusarium moniliforme* which appeared as 80, 60, 80, 40% and 60%, 40, 40, 20 on blotter and PDA, respectively. Grapefruit *(Citrus paradisi)* was found infected with *Dreschslera tetramera, Curvularia lunata,* and *pencillium* spp to the extent of 60, 40, 40 and 60% on blotter paper and 40, 40, 20, 60% on PDA. On lemon *(Citrus limon)* three fungi, *Rhizoctonia solani, Alternaria alternata* and *Aspergillus fumigatus* were identified with the percentage of 80, 60, 40 and 40, 40, 40 on blotter paper and PDA, respectively *Aspergillus fumigatus, Rhizopus* spp, *Rhizoctonia solani* and *Fusarium moniliforme* were identified in 60, 40, 40, 20% on blotter paper and PDA, respectively in rough lemon *(Citrus jambhiri).* Sour orange *(Citrus aurantium)* which was collected from Sargodha was found infected with the extent to 20, 80, 40, 60, 60% and 80, 40, 60, 60, 20% on blotter paper and PDA which have been reported as *Alternaria alternata, Curvularia lunata, Rhizopus* spp, *Rhizoctonia solani* and *Aspergillus niger.* (Table 3.3).

Table 3.3: Comparative study of Citrus Seed Health Testing Method (Sargodha)

Common Name	Fungi Identified	**Sargodha**			
		Blotter Paper		PDA	
		No. of seed infected	%age infection	No. of seed infected	%age infection
Kinnow mandarin	*Aspergillus flavus*	3	60	2	40
	Aspergillus niger	4	80	1	20
	Rhizopus spp.	3	60	2	40
	Penicillium spp.	2	40	2	40
	Alternaria alternata	1	20	1	20
Sweet orange	*Fusarium solani*	4	80	3	60
	Aspergillus flavus	3	60	2	40
	Alternaria alternata	4	80	2	40
	Fusarium moniliforme	2	40	1	20
Grape fruit	*Dreschslera tetramera*	3	60	2	40
	Curvularia lunata	2	40	2	40
	Aspergillus flavus	2	40	1	20
	Penicillium spp.	3	60	3	60
Lemon	*Rhizoctonia solani*	4	80	2	40
	Alternaria alternata	3	60	2	40
	Aspergillus fumigatus	2	40	2	40
Rough lemon	*Aspergillus fumigatus*	3	60	3	60
	Rhizopus spp.	2	40	2	40
	Aspergillus flavus	2	40	2	40
	Fusarium moniliforme	1	20	1	20
Sour orange	*Alternaria alternata*	1	20	4	80
	Curvularia lunata	4	80	2	40
	Rhizopus spp.	2	40	3	60
	Rhizoctonia solani	3	60	3	60
	Aspergillus niger	3	60	1	20

The samples which were collected from Khanpur were infected by *Aspergillus flavus, Aspergillus niger, Rhizopus* spp. *Pencillium* spp and *Alternaria alternata* to the extent of 60, 60, 40, 80, 40% and 40, 40, 40, 60, 40 % on blotter paper and PDA, Respectively, infected Kinnow mandarin *(Citrus nobilis).* Similarly, sweet orange *(Citrus sinensis)* had only four fungi, *Fusarium solani. Aspergillus flavus, Alternaria alternata* and *Fusarium moniliforme* with 80, 40, 40 and 60 %age on blotter paper and with 60, 40, 40, 20 and PDA infection. Grapefruit *(Citrus paradisi)* was found infected with *Dreschslera tetramera, Curvularia lunata,* and *Pencillium* spp with percentage of 60, 40, 60, 80% and 40, 40, 60, 60% on blotter paper and on PDA, respectively. On lemon *(Citrus limon)* three fungi, *Rhizoctonia solani, Alternaria alternata* and *Aspergillus fumigatus* were identified with the percentage of 60, 40, 20 in blotter paper method and PDA method. *Aspergillus fumigatus, Rhizopus* spp, *Rhizoctonia solani* and *Fusarium moniliforme* were identified 40, 80, 60, 40% on blotter paper and 40, 40, 20, 60% on PDA, respectively in rought lemon *(Citrus jambhiri).* Sour orange *(Citrus aurantium)* was infected with *Alternaria alternata, Curvularia lunata, Rhizopus,*app, *Rhizoctonia solani* and with the percentage of 60, 60, 40, 80 and 40, 40, 40, 20, 60 on blotter paper and PDA, respectively (Table 3.4).

Table 3.4: Comparative study of Citrus Seed Health Testing Method (Khanpur)

Common Name	Fungi Identified	**Khanpur**			
		Blotter Paper		PDA	
		No. of seed infected	%age infection	No. of seed infected	%age infection
Kinnow mandarin	*Aspergillus flavus*	3	60	2	40
	Aspergillus niger	3	60	2	40
	Rhizopus spp.	2	40	2	40
	Penicillium spp.	4	80	3	60
	Alternaria alternata	2	40	2	40
Sweet orange	*Fusarium solani*	4	80	3	60
	Aspergillus flavus	2	40	2	40
	Alternaria alternata	2	40	2	40
	Fusarium moniliforme	3	60	1	20
Grape fruit	*Dreschslera tetramera*	3	60	2	40
	Curvularia lunata	2	40	2	40
	Aspergillus flavus	3	60	3	60
	Penicillium spp.	4	80	3	60
Lemon	*Rhizoctonia solani*	3	60	3	60
	Alternaria alternata	2	40	2	40
	Aspergillus fumigatus	1	20	1	20
Rough lemon	*Aspergillus fumigatus*	2	40	2	40
	Rhizopus spp.	4	80	2	40
	Aspergillus flavus	3	60	1	20
	Fusarium moniliforme	2	40	3	60
Sour orange	*Alternaria alternata*	3	60	2	40
	Curvularia lunata	3	60	2	40
	Rhizopus spp.	2	40	2	40
	Rhizoctonia solani	4	80	1	20
	Aspergillus niger	2	40	3	60

Gowder *et al*. (2007) observed that standard blotter method was better for the isolation of large nuber of fungal species. Hence, it was confirmed that blotter paper method showed good result as compared to agar plate method in the present study.

3.3 IDENTIFICATION OF FUNGI

In this study fungi were identified on the basis of colony, hyphae and conidial characteristics. *A. niger* conidia were globose to subglobose in nature, black in color and on PDA, colony was dark brown to black in color *Fusarium solani* conidia have distinct basal foot cell and pointed end and on PDA appeared as cottony pink colored colony. *Rhizopus* was characterized by presence of *Rhizoids* and on pda, colony ws fast growing, cottony, first white becoming grey with age. *A. flavus* was found abundantly on all seeds. Its conidia were globose to subglobose in nature and on age. *Curvularia lunata* has septate conidia and on PDA, gave fluffy black color colony. On PDA colony showed blue green surface pigmentation consisting of dense felt of conidiophore while *Penicillium* conidiophores were branched characterized by presence of brush like structures on PDA, green patches were observed.

3.4 EFFECT OF MYCOFLORA ON SEED HEALTH

To assess the effect of seed-brone fungi on germination of citrus seeds, 15 highly infected citrus seed samples of Kinnow mandarin *(Citrus nobilis),* Sweet orange *(Citrus sinensis),* grapefruit *(Citrus paradisi),* lemon *(Citrus limon)*, rough lemon *(Citrus jambhiri)* and sour orange *(Citrus aurantium)*, were used in the germination test by the rolled towel method. The seed samples, which were highly infected with fungi, showed low percentage of germination and the seed samples, having low percentage of infection showed high percentage of germination. The overall germination percentage was determined which varied between 5-69%. Same pathogens were recorded from rotted seed.

3.5 EFFECT OF FUNGICIDE ON FUNGI OF CITRUS SEED

Citrus seeds were treated with three fungicide i.e., Ridomil Gold, Bavistin and Score and two chemicals salicylic acid and boric acid, to know their effects on seed-borne fungi such as *Aspergillus flavus, Fusarium solani, Aspergillus fumigatus, Curvularia lunata, Rhizopus* spp, *Aspergillus niger* and *Alternaria alternata.*

The seeds were treated individually with the fungicides at different dozes of 20, 30, 40 mg/10 ml and 5, 6, 7 µL/10 mL, respectively and chemical with 20, 30, 40 mg/10 mL. But Ridomil Gold controlled almost all the pahogen at the does of 30 mg/10mL and 40 mg/10 mL.

Only *Aspergillus niger* at 1.0 percent frequency could survive after treatment at 30 mg / 10 mL. But there was no pathogen at 40 mg / 10 mL. Similarly, effect of Bavistin was reported by Ibiam *et al.* (2000) and (2006) on rice seeds. Avistin and Score were also effective at 30 mg / 10 mL and 6 µL/10mL but not as much as Ridomil Gold. Althouh fungicides gave good results at 40 mg / 10 mL.

In chemical application Salicylic acid controlled almost all the patheogen at 30 mg/10 mL and 40 mg / 10 mL. Only *Aspergillus flavus* and *Rhizopus* spp. With 4 percent and 2.5 percent, respectively could survive after chemical treatment at 30 mg / 10 mL. But there were no pathogen at 40 mg / 10mL. Boric acid was also effective at 30 mg/10 mL but not as Salicylic acid at 40 mg/10 mL. Chemical gave good results at 40 mg/10 mL (Table 3.6-3.8).

Table 3.6: Effect of seed-borne Fungi on germination of Citrus seeds (Multan)

Multan	Name of Citrus Seed	Germination %age			Fungi on rotten seed
		Normal Seedling	Ungerminated %age	rotten seed %age	
	Kinnow mandarin	56	9	36	*Alternaria alternata Dreschslera tetramera Fusarium solani Rhizopus spp. Aspergillus niger Aspergillus flavus*
	Sweet orange	72	8	20	*Aspergillus flavus Aspergillus niger*
	Grape fruit	65	8	27	*Alternaria alternate Dreschslera tetramera Curvularia lunata*
	Lemon	58	12	30	*Rhizopus spp. Rhizoctonia solani Curvularia lunata Aspergillus fumigatus*
	Rough lemon	61	13	26	*Rhizoctonia solani Penicillium spp. Aspergillus flavus Alternaria alternata*
	Sour orange	60	7	33	*Alternaria alternate Dreschslera tetramera Curvularia lunata*

Table 3.7: Effect of seed-borne Fungi on germination of Citrus seeds (Sargodha)

Sargodha	Name of Citrus Seed	Germination %age			Fungi on rotten seed
		Normal Seedling	Ungerminated %age	rotten seed %age	
	Kinnow mandarin	65	8	27	*Alternaria alternata* *Dreschslera tetramera* *Fusarium solani* *Rhizopus spp.* *Aspergillus niger* *Aspergillus flavus*
	Sweet orange	63	7	30	*Aspergillus flavus* *Aspergillus niger*
	Grape fruit	58	7	35	*Alternaria alternata* *Dreschslera tetramera* *Curvularia lunata*
	Lemon	59	8	33	*Rhizopus spp.* *Rhizoctonia solani* *Curvularia lunata* *Aspergillus fumigatus*
	Rough lemon	62	8	30	*Rhizoctonia solani* *Penicillium spp.* *Aspergillus flavus* *Alternaria alternata*
	Sour orange	65	7	28	*Alternaria alternata* *Dreschslera tetramera* *Curvularia lunata*

Table 3.8: Effect of seed-borne Fungi on germination of Citrus seeds (Khanpur)

Khanpur	Name of Citrus Seed	Germination %age			Fungi on rotten seed
		Normal Seedling	Ungerminated %age	rotten seed %age	
	Kinnow mandarin	58	7	35	*Alternaria alternata Dreschslera tetramera Fusarium solani Rhizopus spp. Aspergillus niger Aspergillus flavus*
	Sweet orange	62	12	26	*Aspergillus flavus Aspergillus niger*
	Grape fruit	53	13	34	*Alternaria alternata Dreschslera tetramera Curvularia lunata*
	Lemon	67	8	25	*Rhizopus spp. Rhizoctonia solani Curvularia lunata Aspergillus fumigatus*
	Rough lemon	53	12	35	*Rhizoctonia solani Penicillium spp. Aspergillus flavus Alternaria alternata*
	Sour orange	63	9	28	*Alternaria alternata Dreschslera tetramera Curvularia lunata*

CONCLUSION

About 11 fungi including *A. fumigatus* Fres, *A. flavus* Link ex Gray, *Dreschslera tetramera* (Machinney) Sub and Jam, A. *alternata* Nees, *Curvularia lunata* (Wakker) Boed, *Macrophomina phaseolina* (Tassi) Goid, *A. nigar* van Teighem, *F. solani* (Mart.) App and WR, *F. moniliforme*

Sheldon, *Rhizopus and Penicillium* spp were isolated from the seeds of citrus. Out of three fungicide tested i.e., Ridomil Gold, Bavistin and Score, Ridomil Gold showed most significant results against fungicides tested. So, it was concluded by this study that Ridomil Gold can be used for efficient control of fungal diseases in Citrus plants.

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