International Journal of Environmental Science and Toxicology Research (ISSN: 2408-7262) Vol. 3(4) pp. 54-59, April, 2015
Available online http://www.internationalinventjournals.org/journals/IJESTR

Full Length Research Paper

Pathogens identification and charactrization that compromised citrus fruit quality in selected orchards of Sargodha

Amber Khan, Shazia Iram and Anam Rasool*

Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi

Abstract

This study aims to identify and characterize the fungal and microbial pathogens from mandarin fruit of orchards of Sargodha district that are involved in quality degradation of citrus fruit. Sampling was done from orchards of Sargodha Punjab, Pakistan. Out of 57 samples 27 samples were selected from which pathogens were isolated on basis of symptoms of diseases. Potato dextrose agar (PDA) was used as medium for growth of pathogens. The purified cultures of fungal pathogens were then identified and characterized. The identified microbial pathogens include 13 different types of fungi that are *Fusarium sp., Aspergillus sp., Penicillium sp., Curvuleria sp., Alternaria sp., Colletotrichum sp. Guignardia sp.* and Diplodia sp. These pathogens are involved in stem end rot, green mold, blue mold; Fusarium rot, black rot, Post bloom fruit drop, Aspergillus rot, anthracnose and Alternaria brown spot diseases. So it is recommended on basis of present study to conduct further experiments of pathogenecity of these pathogens in future studies to find out the incidence, prevalence and severity of these diseases. Also these pathogens are need to be identified at specie level by using molecular method so that these diseases can be treated and controlled by adopting suitable methods.

Keywords: Orchards of Sargodha, Citrus fruit, mandarin quality, fungal pathogens, diseases.

INTRODUCTION

Citrus is the main fruit tree crop in the world and therefore has a tremendous economical, social and cultural impact in society. Citrus fruit widely used as edible fruits all over the world and it belongs to genus Citrus and family Rutaceae, containing 130 genera in the seven subfamilies with many important fruit and fruit products. It is cultivated throughout the tropical and temperate regions of the world. Citrus fruits include oranges, lemons, limes and grapefruits. Among the Citrus fruits oranges (sweet, mandarin and sour) are the most important as fresh fruit and they contribute to roughly 80 percent of the world's Citrus fruit production (Sidana *et al*., 2013). Pakistan annually produces about 12.0 million tons of fruits and vegetables, of which Citrus fruit is leading in term of production followed by mango, dates and guava. Pakistan is the 10th largest producer of Citrus in the world with 2.29 million tons of production. Punjab is the centre of production and supply of citrus fruits of high quality and grade. Area under different varieties indicates that about 86 per cent of the citrus is covered by Kinnow variety followed by the Musambi (10 %), Feutral (4%) and Red Blood (1%) (Naseer, 2010). Citrus occupies a place of considerable importance in the fruit economy of the country. These are principal source of important nutrients like vitamins C, folic acid, carotenoids, dietary fibers, potassium, selenium and a wide range of phytochemicals that are suggested to be responsible for the prevention of degenerative disease. But there are many environmental factors which are affecting the quality of citrus fruit like environmental conditions, nutrient availability and hormones (Goldschmidt, 1988). Citrus is also susceptible to a large number of diseases caused by plant pathogens. Many serious disease causing pathogens, from bloom to harvesting and post harvesting stage, are involved in

*Corresponding Author Email: anam.rasool32@gmail.com

affecting the production of the crop and considerably deteriorate the fruit quality (Embaby *et al*., 2013). Plant pathogens may infect fruits either before harvest under field conditions or after harvest during transport or storage. The symptoms of diseased infection may be observed at different period after harvest but many pathogens may remain dormant for long period until favorable conditions become available for their development and then leading to visible symptoms. The common postharvest fungi of fruits include *Alternaria spp., Aspergillus spp., Fusarium sp., and Penicillium sp* (Ammar and El-Naggar, 2014).

The objective of this study is to find out the problems related to quality degradation and cosmetic issues of citrus fruit. The study is based on identification of the fungal pathogens that are involved in citrus fruit cosmetic problems and cause quality degradation that makes the fruit unacceptable in international markets. The knowledge provides help in future to find out solution for the problems to prevent the citrus fruit quality which will be very helpful to maintain the export quality of Sargodha citrus fruits and will improve the economy of the country as well.

MATERIALS AND METHOD

The present study was based on identification and characterization of pathogens associated with citrus fruit diseases in selected orchards of Sargodha. 52 Samples were collected from orchards of Sargodha district, Punjab, Pakistan. 'Mandarin' type that is Kinnow (Citrus reticulate) were collected and brought to Mycology and ecotoxicology laboratory, Department of Environmental sciences, Fatima Jinnah Women University, Rawalpindi and isolation, identification and characterization of microbial citrus diseased casual pathogens was done.

Collection of Samples

Sampling of citrus fruit was done from citrus producing orchards of Sargodha district, Punjab, Pakistan. Fruit of mandarin (Citrus reticulate) were selected for isolation of microbial pathogens. Fruits were harvested directly from orchards of Sargodha instead of using stored fruit. 52 damaged samples were collected through random sampling method. Then these samples were brought for lab identification of pathogens causing quality degradation of citrus fruit.

Surface sterilization of Diseased Citrus Fruits: Diseased citrus fruits were then washed in running tap water and with distilled water. Surface sterilization was done with ethanol of 85% concentration for 2 minutes. Then the surface residue ethanol was washed away several times (Bukar *et al*., 2009).

Isolation of Pathogens

The isolation and identification of the causal agents were performed for every single fruit. Fungal pathogens responsible for fruit rotting were isolated from the from the peel surface. After surface sterilization of diseased fruit samples small section from edges of lesions were cut with the help of sterilized surgical blade. The blade was disinfected by putting it into alcohol and the sterilized on hot flame of spirit lamp until it become red hot. Small sections of infected tissue of 2-3mm in length (Parey et al, 2013) were cut at the junction of diseased and healthy portion with the help of disinfected blade after surface sterilizing the sample. These small sections were then transferred to sterilized media plates of PDA. Three small sections were transferred to each plate and then these plates were carefully wrapped with para film and placed into incubator. The inoculated plates were incubated at 25-30°C for 5-7 days and examined daily for the growth of the organism. The growth of fungal colonies were observed and recorded. The cultures thus obtained were then subjected to purification (Parey *et al*., 2013).

Purification of Pathogens

Fungal cultures obtained were then purified by 'single spore isolation method'(Choi, Hyde and Ho, 1999). These pure cultures were maintained on PDA slants for further study.

Identification of Pathogens

The fungal cultures were identified at genus level on basis of macroscopic characteristics like colony morphology, color, texture, shape and appearance and microscopic characteristics like conidia shape, hyphe color, septation, concentric zone, pigmentation, fruiting bodies or any other visible structures by observing under compound microscope at magnification of 10X and 40X (Navi *et al*., 1999).

RESULTS

Different types of fungal pathogens were isolated from diseased citrus fruits. This study identified 13 fungal isolates which were associated with diseased citrus fruits from Sargodha orchards. The Results showed that four Aspergillus species, one Alterneria spp., one Curvularia spp., three Fusarium, one Penicillium spp., Guignardia citricarpa, colletotrichum spp., Diplodia spp., were

involved with citrus fruits quality degradation in Sargodha district, Punjab, Pakistan.

Sampling Area

Sargodha district produces the largest amount of citrus in Pakistan. Most of the kinnow production and processing industry fall in this district and high quality citrus are export to international market. That is why samples from Sargodha region were selected, where largest amount of citrus is grown for the past many decades.

Observed Symptoms on Fruit Samples

Physically observation of diseased fruits showing blemishes, blisters surrounded by yellow halos, dark brown to black lesions, hard spot (shot-hole spot), speckled blotch, freckle spot, Decay proceed through rind, Producing fingerlike projections of brown tissue. Table 1.

Identification and characterization of fungal pathogens if citrus fruits

Isolation was made from the infected fruit samples showing typical symptoms of diseases of citrus fruit, collected from orchards of Sargodha, Punjab Pakistan. 13 different isolates were obtained which include three species of *Fusarium*, four species of *Aspergillus* species, one species of *Diplodia*, *Colletotrichum*, *Alternaria*, *Penicillium*, *Guignardia* and *Curvularia*. The pure cultures of these isolates were maintained by single spore method on potato dextrose agar (PDA) medium. The cultural morphological and microscopic characters of these isolates on PDA were observed visually and under microscope and are described in table 2 and figure 1.

DISCUSSION

This study showed that numbers of fungi were associated with the loss of citrus fruits due to its cosmetic issues in Sargodha orchards of Punjab, Pakistan. The isolation results of this study indicated that number of fungi of genera *Aspergillus, Alterneria, Curvularia, Fusarium, Penicillium, Guignardia, Colletotrichum* and *Diplodia* were involved in quality degradation citrus fruits of Sargodha district having cosmetic problems. Many diseases of citrus fruits were described worldwide by many researchers and these diseases were given colorful and descriptive names such as blue mold, green mold, gray mold, brown rot, black spot, black rot, black pit etc. *Aspergillus niger* was found to be associated with deterioration of orange.

Aspergillus spp. is the predominant organism associated with the spoilage of citrus fruit. Black mould caused by *Aspergillus niger* resulted post harvest spoilage in of citrus fruit and spoilage of acid lime at field (Anitha *et al.*, 2014). The most important postharvest disease of fruits worldwide is caused by *Penicillium digitatum*, *Aspergillus niger* and *Fusarium spp.* In study of Singh et al. (2012) *P. digitatum, A. niger and Fusarium spp*. were originally isolate from citrus fruits which were identified by using the taxonomic and morphological references. Peever et al. (2005) reported that *Alternaria spp*. cause several diseases of citrus, including *Alternaria* brown spot, leaf and fruit spot and black rot of fruit of several Citrus species. *Alternaria* black rot of citrus fruit is a significant postharvest problem that appears in the field prior to harvest. Black rot causal agent was first identified as *Alternaria citri* . *Alternaria citri* also has been identified from various healthy citrus fruit, leaf and twig tissues. It was also reported that citrus black rot was caused by more than one morphological species of *Alternaria* like *Alternaria alternata.* The study of Ezeibekwe et al. (2008) report ed that *Colletotrichum* and *Fusarium spp* as specific pathogens causing damping off diseases of Citrus. Also reported that blue and green fruit molds diseases of Citrus are caused by *Penicillium spp* and *Botryodiplodia theobromae*, *Aspergillus spp* and *Penicillium spp* were reported as the most important fruit rot pathogens of Citrus. *Lasiodiplodia* stem-end rot is an important disease of citrus fruit in warm and humid citrus growing regions such as Florida and Caribbean reported by Fischer et al (2009). *L. theobromae* is fungus that cause stem end rot that infects the fruit at the stem-end of the fruit, leading to developrnent of soft brown to black decay symptoms at both fruit ends. While that of Anthracnose was caused by *Colletotrichum spp.* That generally attacked on injured fruits. However, anthracnose symptoms can be observed even in non-injured fruits of citrus varieties. In Florida, anthracnose is a major cause of decay in Tangerines type. Black spot of citrus caused by *Guignardia citricarpa* was first officially noticed in Australia in 1895 on fruits in the citrus-growing areas around Sydney. Black spot of citrus is a serious disease of citrus cultivars in countries like Australia, Guangdong province in China and South Africa. This is why *G. citricarpa* is considered to be the most important pathogen of citrus in China, Australia and South Africa. (CABI and EPPO, 2013). *Curvularia lunata* was isolated from selected stored citrus fruit in India reported by Ghurde and PachKhidi (2010) that was involved in citrus fruit spoilage. All the identified fungal pathogens need to be identified at species level so that proper solution for problem can be applied in future.

Table 1. Showing the Symptoms of Diseases on Citrus Fruit Samples of Sargodha Orchards

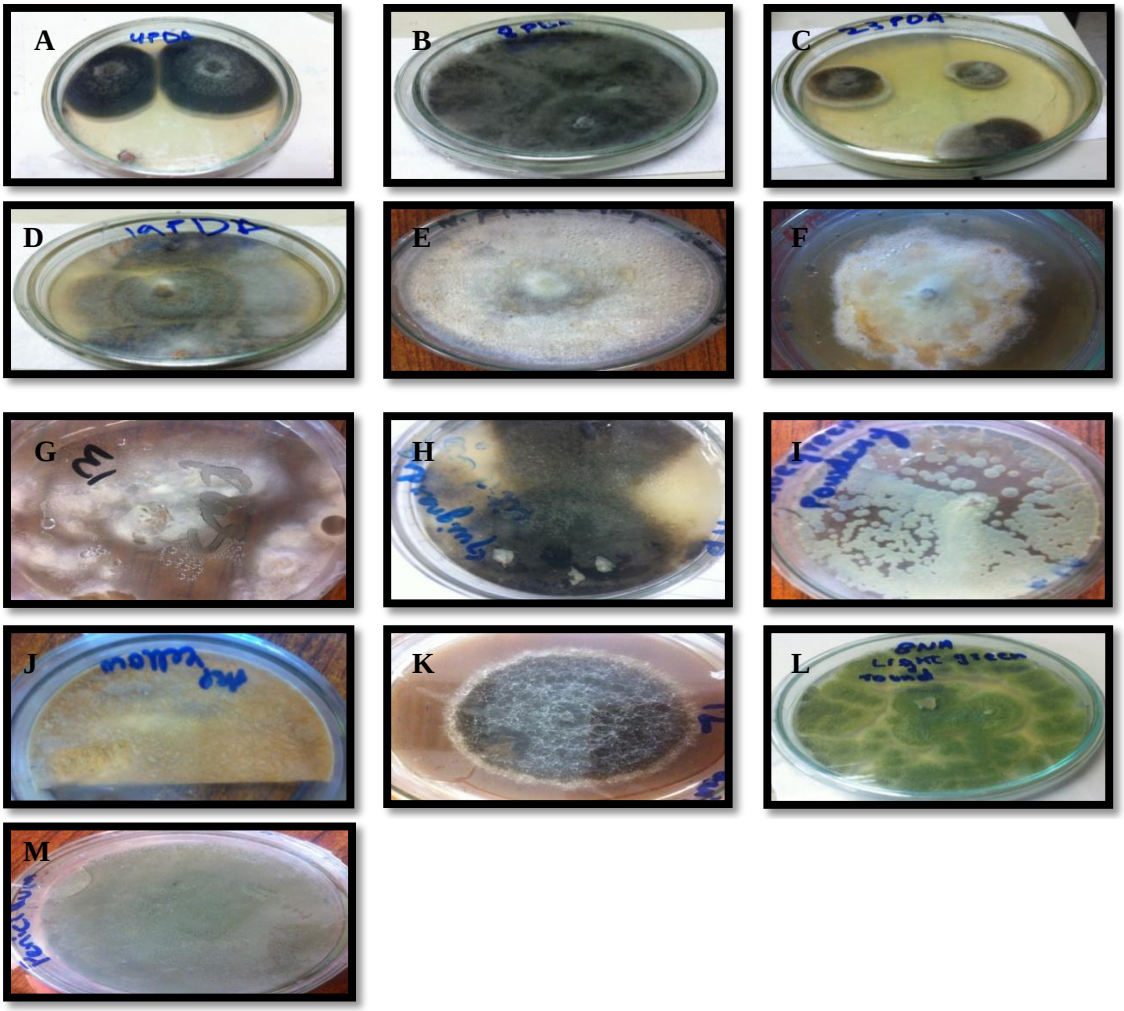
PICTURES OF FEW SAMPLES SHOWING SYMPTOMS		
 Blemishes	 Blisters surrounded by yellow halos	 Decay proceeds through rind, producing fingerlike projections of brown tissue.
 Dark brown to black lesions	 Hard spot (shot-hole spot), speckled blotch, freckle spot.	

Table 2. Colonial and Morphological Characteristics of Fungi Associated with the Spoilage of citrus fruits

Diseases	Isolates	Colony Characteristics	Spore Characteristics	Colony diameter (cm)	Spore size (µm)
Black mold rot	*Aspergillus niger*	Black color colony with white or yellow basal felt growth produces radial fissures in the agar.	Sporangiophores were prominent under microscopes with short circular to semicircular shape; the spores were dark and oval in shape.	7.5	Length= 19-30 Width= 3.5-5
Green mold rot	*Aspergillus flavus*	Colony is powdery yellow green with cottony, rapid growth with granular, flat mycelium	Hyphae were branched & septate, conidia were greenish, globose to subglobose with rough surface & occur in chains of two or more	8.8	Length= 6-8 Width= 4-5
Aspergillus rot	*Aspergillus sp.*	Colony is yellow orange in color or ochraceous or buff with restricted growth	Conidia were yellow gold in color with globose to slightly ellipsoidal, smooth walled	3.1	Length= 14.7-27 Width= 6-9
Damping off	*Aspergillus fumigates*	Blue-green to gray colony color with powdery texture	Conidiophores was clavate vesicle, thick walled with Conidia of Smooth, globose to subglobose	6.0	Length= 19-26 Width= 4-6
Blue & Green mold	*Penicillium sp.*	Colony was fast growing bluish green in color with powdery texture	Conidia are globose greenish with smooth wall	8.6	Length= 11-30 Width= 7-25
	Fusarium sp.	Mycelia of the isolates were delicate, white to creamy color, with margins slightly lobed or smooth	Spore are oval to reniform and without any septation	6.8	Length= 36.2-54.3 Width= 21-36
Fusarium rot	*Fusarium sp.*	Whitish cream color colony	Spores were curved, short, with blunt apical and pedicellate basal cells	4.0	Length= 54-126 Width= 27-63
	Fusarium sp.	Sparse white aerial mycelium	Conidia were sickle-shaped to almost straight	3.8	Length= 54.3-72.4 Width= 28.96-36.2

Table 2. Continue

Anthracnose/ dieback	*Colletotrichum sp.*	White to grey colony with circular shape and fluffy mycelium. Conidia appear in masses with pale buff in color.	Spores were sub cylindrical with round and smooth end	8.0	Length= 40.7-54.3 Width= 13-18.1
Black rot	*Alternaria sp.*	Dark brown blackish,	Conidia were ellipsoidal with long cylindrical beak, brown in color with septation	7.5	Length= 23-28 Width= 8-15
Stem end rot	*Diplodia sp..*	Grey to black color colony with shiny, black pycnidia which were stromatic, globose & ostiolate	Spores were dark brown in color, bicelled and thick walled.	8.9	Length= 18-37 Width= 10-18
Citrus black spot	*Guignardia citricarpa*	Colonies are dark brown to black and the mycelium was thick and prostrate.	Conidia were obovate to elliptical, hyaline, nonseptate, multiguttulate with a colourless appendage	5.6	Length= 226-271 Width= 81.45-90.5
Dieback	*Curvularia sp.*	Colony is rapidly growing with olive brown to black in color when colony is mature	Hyphae are septate and brown, conidia is curved and septate with dark to pale brown in color	6.4	Length= 48.8-63 Width= 32-36



Figures 1. Isolated fungal pathogens on PDA media
(a)*Curvularia* sp.,(b) *Diplodia* sp., (c) *Alternaria* sp.,(d) *Colletotrichum* sp., (e) *Fusarium* sp., (f) *Fusarium* sp., (g) *Fusarium* sp., (h) *Guignardia* sp., (i) *Aspergillus fumigates,* (j) *Aspergillus* sp., (k) *Aspergillus niger,* (l) *Aspergillus flavu,*(m) *Penicillium* sp

CONCLUSION

The present study concluded that Citrus is the important food crop of Pakistan that contributes a huge economic share to the country's economy. Sargodha district in Punjab, Pakistan is the main and major area of production of Kinnow (*Citrus reticulate*) which is exported to national and international markets. Now production of citrus fruit is declining in country because of different problems and diseases of citrus which also affect the citrus fruit quality and make them unmarketable due to cosmetic issues. In this study lab identification of fungal pathogens was made related to citrus fruit quality degradation. Fungal pathogens were isolated on basis of different blemishes and lesions present on fruit samples of Sargodha Orchards. The cultures obtained were then purified on Potato Dextrose Agar (PDA) media. The purified cultures of fungal pathogens were then identified and characterized on genus level through morphological characteristic. The identified microbial pathogens include thirteen different types of fungi including *Fusarium* spp*., Aspergillus* spp*., Penicillium* sp*., Curvuleria* sp*., Alternaria* sp*., Colletotrichum* sp*. Guignardia* sp*.* and *Diplodia* sp*.* These pathogens were found to be associated with the citrus fruit of Sargodha orchards and were found to be involved in many serious citrus fruit diseases thus deteriorating the quality of citrus fruit, cause serious damage to the citrus fruit and compromised the export quality of citrus fruit as well.

RECOMMENDATION

On the basis of present study following recommendations are proposed that in future further study can be carried out with pathogenicity test of these fungi on citrus species so that the problems caused by them can be found out. Further experiments can be set up regarding incidence, prevalence and severity of these diseases. To prevent the citrus fruits from all these pathogens these pathogens can be identified at species level by molecular methods. Awareness about the spread of these diseases can be given to the farmers and orchard owners so that these diseases of citrus fruit can be controlled at source level. After identification of problems and their causes it will be possible to find out the solution for these problems. So that problems can be treated and managed by adopting appropriate methods.

ACKNOWLEDGMENT

The research was conducted as part of a grant supported by the Government of Australia through ACIAR under Pakistan-Australia Agricultural Sector Linkages Program (ASLP). This financial support is greatly appreciated.

REFERENCES

Ammar MI, El-Naggar MA (2014). Screening and Characterization of Fungi and their associated Mycotoxins in some Fruit Crops. *International Journal of Advanced Research*. 2(4): 1216-1227.

Anitha M, Swathy SR, Venkateswari P (2014). Prevalence of disease causing microorganisms in decaying fruits with analysis of fungal and bacterial species. *Int. J. Res. in Health Sciences*. 2(2): 547-54.

Bukar A, Mukhtar MD, Adamu S (2009). Isolation and identification of postharvest spoilage fungi associated with sweet oranges (*citrus sinensis*) traded in Kano metropolis. *Bayero Journal of Pure and Applied Sciences*. 2(1): 122 – 124.

CABI and EPPO (2013). Guignardia citricarpa. Data Sheets on Quarantine Pests. *EPPO Quarantine Pests.*

Choi YW, Hyde KD, Ho WH (1999). Single spore isolation of fungi. *Fungal Diversity*. 3: 29-38.

Domsch KH, Gams W, Anderson TH (1998). Compedium of Soil Fungi. Academic Press., London. 860.

Embaby EM, Hazaa M, Hagag LF, Ibrahim TE, Abd El-Azem FS (2013). Decay of Some Citrus Fruit Quality Caused by Fungi. *Journal of Applied Sciences Research*. 9(11): 5920-5929.

Ezeibekwe IO, Onuoha CI, Mbagwu FN, Nwachukwu PC (2008). Studies on Soil Pathogens Associated with Citrus Disease. *International Science Research Journal*. 1(2): 165 – 168.

Ezeibekwe IO, Unamb CI (2009). Pathogens Associated with Citrus Fruits Rots in Imo States, Nigeria. *Journal of America Science*. 5(4): 129-132.

Fischer IH, Ferreir MO, Spósito MB, Amorim L (2009). Citrus postharvest diseases and injuries related to impact on packing lines. *Scientia Agricola*. 66(2): 21 0-217.

Ghurde MU, PachKhidi AU (2010).The market and storage diseases of fruits from Amravati (Maharashtra). *India Bionano Frontier*. 3(2).

Goldschmidt EE (1988). Regulatory aspects of chloro-chromoplast inter conversions in senescing Citrus fruit peel. Isr. J. Bot. 47:123-130.

Gordh G, Mckerdy S (2013). The Handbook of Plant Biosecurity: Principles and Practices for the identification, containment and control of organisms that threaten agriculture and environment globally. Invasive Pathogens in Plant Biosecurity, Case Study Citrus Biosecurity. *Springer Netherlands*. 547.

Naseer M (2010). Export of kinnow. Report by trade development authority of Pakistan government of Pakistan, Faisalabad.

Navi SS, Bandyopadhyay R, Hall AJ, Bramel-Cox PJ (1999). A pictorial guide for the identification of mold fungi on sorghum grain. *International Crops Research Institute for the Semi-Arid Tropics.* 118.

Parey MA, Razdan VK, Sofi TA (2013). Comparative study of different fungi associated with fruit rot of chilli and screening of chilli germplasm against Colletotrichum capsici. *International Journal of Agricultural Crop Sciences*. 5(7): 723-730.

Peever TL, Carpenter-Boggs L, Timmer LW, Carris LM, Bhatia A (2005). Citrus Black Rot is Caused by Phylogenetically Distinct Lineages of Alternaria alternate.*The American Phytopathological Society*. 95(5).

Pryor B, Matheron M, Figuli P (2008). Characterization of Alternaria isolates associated with Alternaria Rot of Citrus. Citrus Research Report, the University of Arizona College of Agriculture and Life Sciences.

Sidana J, Saini V, Dahiya S, Nain P, Bala S (2013). A Review on Citrus – "The Boon of Nature". *Int. J. Pharmaceut Sci.*18(2): 20-27.

Singh H, Al-samarai G, Syarhabil M (2012). Exploitation of Natural Products as an Alternative Strategy to Control Postharvest Fungal Rotting of Citrus. *International Journal of Scientific and Research Publications*. 2 (3).

Wardowski WF, Nagi S, Grieson W (1986). Fresh citrus fruits. Avi publishing. 571.

How to cite this article: Khan A, Iram S, Rasool A (2015). Pathogens identification and charactrization that compromised citrus fruit quality in selected orchards of Sargodha. Int. J. Environ. Sci. Toxic. Res. Vol. 3(4):54-59