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Isolation and Identification of Post-Harvest Fungal Pathogens of Spoilt Grape Fruits

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ABSTRACT

The present investigation was carried out with survey, isolation of the pathogens responsible for post harvest spoilage, their pathogenicity and symptomatology of fungi in order to suggest suitable control for management of post harvest diseases of grapes. Present investigation showed that, the extent of losses due to post harvest diseases were lesser in the field as compared to post harvest losses in marketplace. Among the post harvest diseases, fungus *Fusarium oxysporum, Rhizopus*, *Aspergillus niger*, *Penicillium*, *Phomopsis, Pestalotiopsis* and *Botryodiplodia* were the major pathogens isolated from infected grape, which later on leads to rot. the results of the present study have revealed the spoiled grapes were mainly contaminated with *Fusarium oxysporum, Aspergillus niger* and *Penicillium* sp, and least infection with *Rhizopus*, *Phomopsis, Pestalotiopsis* and *Botryodiplodia*. These fungal pathogens could be harzadous to human health, due to some of these pathogens could produce mycotoxins which might lead to economic loss, health hazards and decrease the quality and quantity of grape fruits. Appropriate post-harvest proccing and intensive care during harvesting season will be helpful to reduce the infection and contamination with these fungal pathogens and for expanding the shelf life of gape fruits against fungal pathogen during storage period.

KEYWORDS: *Isolation, Identification, Grape, postharvest diseases, spoilage and postharvest losses*

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INRODUCTION

Grape (Vitis vinifera L.) belonging to family Vitaceae is one of the most commercially important tropical fruit crops worldwide. Spoilage fungi can reduce the shelf life of fresh fruits and cause economic losses by lowering quality (Chul Kwon 2018). The post-harvest disease is one of the most prevalent and common disease, which could lead to drop and decline in quality of ripped fruits by shortening their storage period. Low pH, higher moisture and nutrient contents of fruits make them suitable environmental conditions to attack by pathogenic fungi, which cause decaying and make them unacceptable for consumption and marketable place. Postharvest disease have an impact on food security, quality and economic development. Postharvest losses may occur during postharvest handling, pathogens infection, storage, preparation, packing, transport and distribution, which decreases the quality, quantity and market value of agricultural commodities (kader 2005, Parfitt *et al* 2010). Fruits contamination by microorganisms may occur through direct contact with soil, dust, water and during postharvest processing. Moreover, the surface of these fruits is prone to damage during harvesting and postharvest handling (Chul Kwon 2018). In the developing countries the losses are always higher because harvest and postharvest operations to protect grapes from mechanical damage are very poor or completely absent (FAO, 2005). Several species of fungi, belonging to the genera *Botrytis, Cladosporium, Alternaria, Pestalotiopsis, Fusarium, Penicillium, Stemphylium, Rhizopus* and *Aspergillus*, were isolated from rotten grape harvested from fields and markets in different localities (Ling, 2008). *Cadophora luteo-olivacea* was isolated from a brown skin spot developed on the berries of Japanese grape during storage at 0 °C for 3 months (Nakaune, 2016). The *Pestalotiopsis* sp was one of the prevalent and common fungi isolated from canes, woody tissue, berries, flowers, and leaves of grapevines has also been reported (Pando et al. 2001, Sergeeva et al. 2005, Urbez-Torres et al. 2009 & 2012). Several fungi such as *Botrytis*, *Alternaria*, *Pestalotiopsis*, *Penicillium*, *Stemphylium* and *Rhizopus* were isolated from rotted berries of grapes (Xu et al, 1999). *P. uvicola* has been reported to cause fruit rot disease of grape (Ryu et al. 1993), However, The presence of the fungi is most likely originated from the farms where the fruits were cultivated and harvested. The most spoilage organisms may be present on fruits from the farm, during harvest operations, and this may result in post-harvest contamination and spoilage of these fruits (Jay, 2003). Some moulds may produce mycotoxins and others could cause infections or allergies. Both, toxigenic and pathogenic fungi have been isolated from spoilt fruits (Stinson et al., 1981, Tournas and Katsoudas 2005). Botrytis cinerea, the cause of gray mold, is considered one of the most important postharvest decays of fresh fruit and it is still capable of causing considerable postharvest losses (Droby and Lichter 2004, Feliziani and Romanazzi, 2014, Elad et al., 2015).

MATERIALS AND METHODS

Fruit materials

The assess of losses in grape fruits caused by fungal diseases during post-harvest was performed between the field as compared to marketplace. About 400 samples of infected

fruits were purchased from different local fruits. The samples were sorted to identify infected grapes, which were then transferred into sterile polythene bags, labeled, and assessed in the laboratory. The loss due to fungal diseases was assessed at weekly intervals for three months.

Isolation of fruit spoilage fungi

Upon arrival at laboratory and before the isolation process. Petri plates, surgical blades media bottles and distilled water, were sterilized in the autoclave at 121˚C for 20 minutes and then dried at 90˚C in hot air oven. Sample of fungus was isolated from infected fruits. The fruits were washed with sterile distil water and then sub-culturing the fungi washed off water. The sub-culturing was carried out by using a sterile fresh medium of potato dextrose agar (PDA) and incubated at 28°C for 5-7 days until fungal proliferation on medium surface. The isolation of pure fungal colony in culture medium was performed by using slants of a sterile fresh medium of PDA and incubated at 28°C for 5-7 days. The isolated fungi were maintained at 4°C (Iniekong et al, 2015).

Pathogenicity of the Isolates on grape Fruits

All the isolates in the present study were tested for pathogenicity on the fresh grape fruits. Two groups of treatments were designed. The first group experiment (1), The fruits were washed with sterile distil water and then wounded once to a depth of 3 mm with a sterilized needle in the equatorial zone (wounded fruits) and The second group experiment (2) The fruits were washed with sterile distil water and non-wounded (intact fruits). A 5-mm-diameter plug from a 5-day-old mycelial culture of isolates was inoculated onto intact and wounded grape fruits. PDA was used as the untreated control treatment. Thereafter, all the treatments fruits were incubated at room temperature (20 - 25 °C) and all the experiments were duplicated twice The parameter of disease incidences was measured at 5 days after inoculation . The pathogenicity was determined as the ability to cause the typical decay symptom, and the number of fruit infected (Zhang et al 2019).

Identification of the isolated fungi

Pure colonies of fungal isolates were classified according to the conventional guidelines of fungus (Ellis 1971, Barnett & Hunter 1972, Watanabe 2002, Gilman 2008, Samson and Varga, 2007).. The pathogenicity of the isolated fungal species was confirmed by inoculating them in 250 ml Erlenmeyer flasks containing 5% fresh uninfected fruits peels under aseptic conditions, to induce rotting. The inoculated flasks were incubated at 28°C in a rotary incubator shaker with shaking at 150 rpm for five days. The pathogens were re-inoculated after isolation onto healthy fruits (Tomkin & Trout 1931).

RESULTS AND DISCUSSION

The studies showed that the commonly fungal species isolated from the infected grape fruits at all stages were identified as follows *Fusarium oxysporum, Rhizopus*, *Aspergillus nige*r, *Penicillium*, *Phomopsis, Pestalotiopsis* and *Botryodiplodia* (Table1), (Figure1,2&3). Fruits were susceptible to infection with a number of diseases from the seedling to the fruits (Alemu, 2014 and Palejwala et al., 1987). In post-harvest conditions, grapees get infected by various fungal diseases like, *F.oxysporum* was isolated from roots of declining grapevines growing in the glasshouse with material obtained from roots of field grown vines (Highet and Nair 1995). *Aspergillus niger* is a fungus commonly found on grapes (Chulze, 2006). However, The *Pestalotiopsis* sp and *Botryodiplodia* sp were prevalent and common fungi isolated from rotted grape and caused major losses to grape. However, Several postharvest fungal diseases limit the period of storage and marketing of grapes and present a major indices that causes serious economic losses Fungi have been identified all over the world that cause rotting in fruits and reduce their nutritional value, medicinal value and storage period (Karabulut et al. 2002, De Cal *et al* 2009).

Table1. Symptoms of post-harvest fungal diseases in grape

Fruit	Pathogen	Disease	Symptoms
Grapes	*Aspergillus niger*	rot	light-brown circular spots, that enlarges into darker lesion
	Fusarium oxysporum	rot	brown discoloration, but quickly turn darker brown lesion later
	Penicillium sp.	rot	Watery spots, changes into bluish green at later stages
	Rhizopus stolonifer	rot	Water soaked lesions, soft decay
	Phomopsis sp	rot	Brown to black spots
	Botryodiplodia sp	rot	Black turn darker brown lesion later

Figure1: Infected grape fruits

Figure2: *Aspergillus niger* grown on grape fruits

Figure3: *Fusarium oxysporum* grown on grape fruits

CONCLUSION

The deterioration of most fruits is caused by fungi infection, Grape is highly perishable fruit and the post-harvest losses in grape fruits being one of the major constraints all over the world. the results of the present investigation have revealed the spoiled grapes were mainly contaminated with *Fusarium oxysporum, Rhizopus*, *Aspergillus niger*, *Penicillium*, *Phomopsis, Pestalotiopsis* and *Botryodiplodia*. Most of the fungi isolated were observed to be able to reinfect healthy grapes within short period and most grapes were observed to have been infected with more than one fungal species. the spoiled grapes were mainly infected with *Fusarium oxysporum, Aspergillus niger* and *Penicillium* sp, and least infection with *Rhizopus*, *Phomopsis, Pestalotiopsis* and *Botryodiplodia*. Moreover, post harvest diseases were lesser in the field as compared to post harvest losses in marketplace. Appropriate post-harvest proccing and intensive care during harvesting season could reduce the infection and contamination with fungal pathogens that might be harzadous to human health, due to some of these pathogens could produce mycotoxins which might lead to economic loss, possible health hazards and decrease the quality and quantity of grape fruits. This study will be great helpful for expanding the shelf life of gape fruits through the appropriate control management against contamination and infection by fungal pathogen during storage period.

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CONFLICTS OF INTEREST:

The authors declare no conflict of interest.

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