



RESEARCH PAPER

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Effect of incubation temperature on lesion diameter of *Penicillium expansum* on apple fruit varieties

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Abstract

Current research work was conducted to determine impact of various genotypes and heat on shelf life of apple. Study was carried out *in vitro*. Effect of temperature on lesion diameter of fungal growth in most prominent apple fruit varieties growing in Pakistan was assessed. Four temperature (5, 15, 25 and 35°C) treatments were provided to fruits of each variety after inoculation with spore suspension adjusted at 10⁶spores/ml and growth of *P. expansum* was measured for 12 days at 3 days interval. Pathogen showed maximum growth (23mm) at 25°C while minimum (11mm) at 5°C in all varieties and moderate growth was observed at 15°C and 35°C. Golden delicious apple which have thin cuticle and prone to injuries more frequently as compared to other varieties was found most susceptible at all temperatures while Red delicious proved to be the most resistant against growth of pathogen at all temperature sets comparatively. It was concluded that Red delicious is most resistant against pathogen studied consequently can be saved for longer time without providing extraordinary conditions while Golden delicious apple should be kept in special environment to reduce damage of product after harvesting due to its most susceptibility against blue most disease.

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Introduction

Apple is the most widely cultivated fruit in the world and also called sweet gold of Pakistan. The total area, reported under apple cultivation in Pakistan is about 110.16 thousand hectares with a production of 598.3 thousand tons (MINFA, 2011-12). In Pakistan number of apple varieties are grown like Roya Gala, Amri, Kashmiri Amri, Golden Delicious, Red Delicious, Sky spur, Banki, Kulu etc. Low chilling varieties such as Anna, Summer Gold and Golden Dorset are prominent. Fungal infections are the major cause of postharvest rots of fresh fruit and vegetables in storage, transport causing considerable economic losses in the commercialization phase (Gatto *et al.*, 2011). Principal causes for post harvest losses are decay, external damages incurred during harvest and handling, and harvest at an improper maturity stage (Thorne and Alvarez., 1981). Infections caused during postharvest conditions mitigate the shelf life and shape the market value of fruits negatively (Tripathy *et al.*, 2008).

Apple being a delicate product is prone to qualitative and quantitative losses after harvest. The losses may occur during postharvest operations or storage which could be as high as 17% (Shah *et al.*, 2002) or even greater. Postharvest fruit rot diseases, left hysterical, can damage accessibility of apples significantly during the postharvest storage period which can last for 12 months (Ilyas *et al.*, 2007). A survey for postharvest diseases of apples conducted in Punjab (Pakistan) revealed prevalence of blue mold, Mucor rot, Sphaeropsis rot, Bull's Eye rot and Gray Mold. Blue mold caused by *Penicillium expansum* Link was found with highest incidence (56%) (Sattar, 2014).

Blue mold is one of the most important reasons of postharvest losses in apple production in Brazil (Brasil, 2005; Coelho *et al.*, 2007). Moreover, mycotoxins the secondary metabolites produced by molds have unsympathetic effects on humans and animals (Zain, 2011). The contamination of fruits with mycotoxins has not only caused health risks but also resulted in financial losses, particularly for exporting countries (Fernandez-Cruz *et al.*, 2010).

Temperature and humidity are considered the major abiotic factors determining the potential for germination and propagules growth of fungal spores on the fruit surface (Maganand Lacey, 1988; Plaza *et al.*, 2003). *In vitro* studies on *P. expansum* showed the radial growth rate of fungus positively correlated with the incubation temperature and water activity of medium (Lahlali *et al.*, 2005; Lahlali, 2006). However the effect of temperature on the growth (lesion diameter) of *P. expansum* was never studied on apple fruit in Pakistani apple varieties. Therefore the main objective of the present study was to determine effect of incubation temperature on *in vivo* growth of *P. expansum* on apples fruits of three varieties of Pakistan.

Materials and methods

Isolation of Fungus and Spore Suspension Preparation

Penicillium expansum was isolated from decayed apple fruits collected from fruit storage houses and fruit markets in Punjab, pathogenicity test was performed for confirmations. For long-term storage, the isolates were preserved on silica gel under optimum conditions for further use. Isolated pure culture was used for making conidial suspension. Conidial mass was washed with sterilized distilled water and concentration was adjusted at 10^6 spores/ml by using haemocytometer

Fruit Preparation

Healthy fruits were selected and sterilized with 1% sodium hypochlorite by dipping them for two minutes and then rinsed twice with sterilized distill water. After rinsing fruits were left for an hour for drying. Each fruit was wounded at equatorial sites with the help of cork borer having 3mm width and 6mm depth.

Temperature Adjustments

Four incubators (Ilshin America, CI BB-012) were maintained at 5°C, 15°C, 25°C and 35°C and fruits were placed in them in trays in such a way that proper aeration can be maintained. Eight fruits were treated for each temperature study and were inoculated with 10µL conidial suspension and kept in separate incubator.

After treating apples with the spore suspension they were kept in incubators maintained at different temperatures mentioned. Observations were taken for 12 days at interval of 3 days.

Statistical analysis

Statistical software SPSS v 16.0 was used to analyze data; two types of tests were used to check the effect of above mentioned sets of temperature on growth of the fungus in 12 days from incubation date.

Results and discussion

At 5°C fruits of Golden Delicious and Royal Gala showed significantly higher growth of pathogen as compared to Red Delicious (Fig.1) while at 15°C Golden Delicious showed more significant growth (28mm) as compared to other varieties but overall fungal growth was retarded as compared to other high temperature in study.

25°C temperature was found the most favourable temperature for fungal growth and Golden delicious showed highest lesion diameter (36 mm) while other two varieties were also significantly affected and expressed high growth rates (Fig.1) as compare to their other temperature treatments similar results were found by Lahlali *et al.*, in 2005 that the highest growth rate of *P. expansum* was observed *in vitro* conditions at 25°C while humidity was found non significant. At 35°C growth of pathogen became slow and all varieties showed less lesion diameter (Fig.1) and similar results were reported from model developed by Katleen Baert *et al.* in 2007 that the growth of *P. expansum* starts retarding at 30°C.

This study depicts that fungal growth was greatly retarded at 35°C as compared to other lower incubation temperature sets.

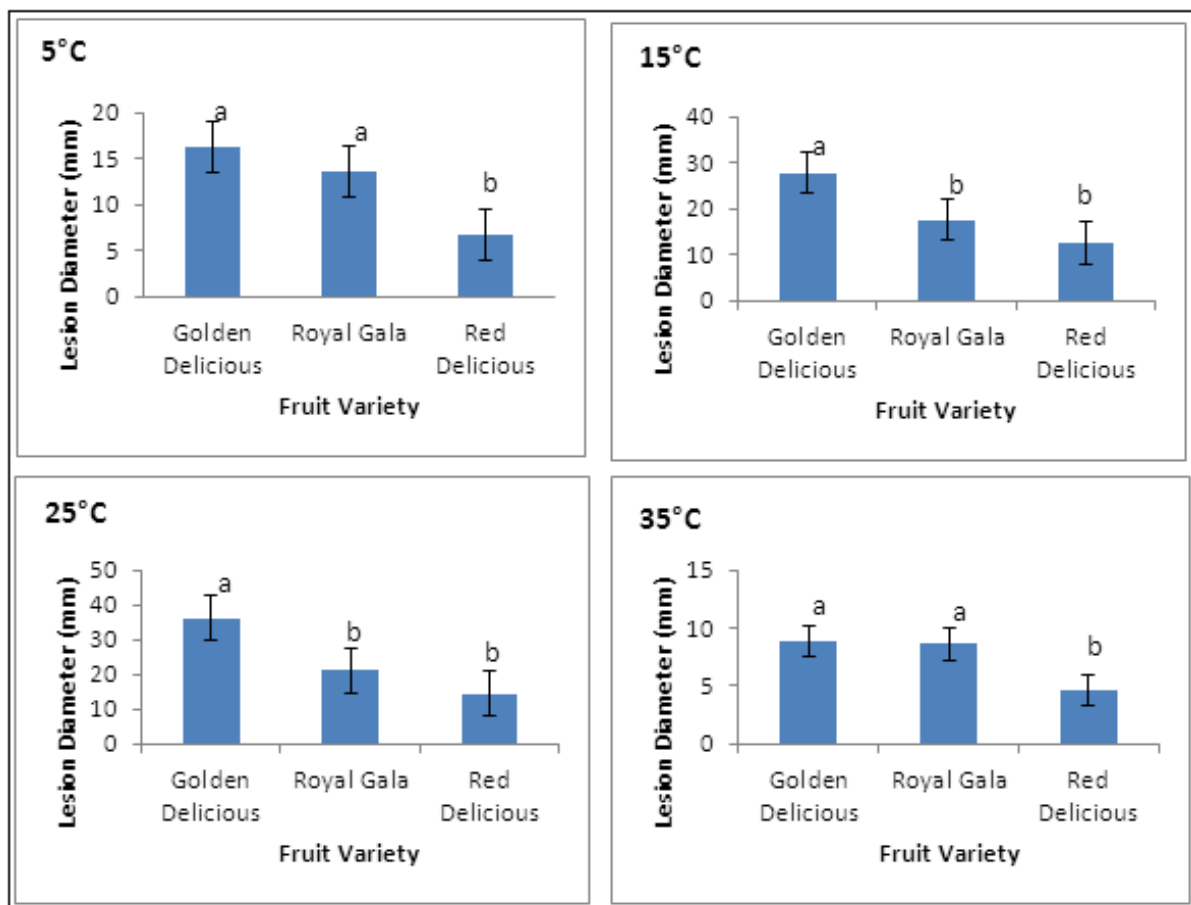


Fig. 1. Lesion diameters (mm) of *P. expansum* in three apple varieties obtained at various treatments of incubation temperature 5, 15, 25 and 35°C. Treatments having the same letters are not significantly different according to Duncan's multiple range test at ($P < 0.05$).

Each variety showed fungal infection with varying degree as Pansnen *et al.*, (1990) found that environmental factors like temperature and air humidity could not stop germination of spores of *P. expansum* and even a small favourable time lap can start fungal growth and cause infection. With the increase in temperature fungal growth increased and found maximum (36mm, Golden delicious) at 25°C while the lowest (7mm) at temperature at 5°C (Red Delicious) (Fig. 1).

Furthermore, it was also observed that fungal infection increased with the increase of incubation temperature up to 25°C and then decreased with the further increase in temperature (35°C). On the basis of sensitivity and resistance significant differences were observed among different apple varieties (Watkins and Rosenberger, 2002) as this study revealed that Golden Delicious was more sensitive to *P. expansum* (blue mold) as compared to other two varieties. Golden delicious apple have thin cuticle, least amount of acids and are prone to more injuries as compared to other varieties (Jan *et al.*, 2012) that may provide more chances of pathogen contact and fungal growth. Furthermore the carbohydrates, protein, fat and fiber contents are high in Golden Delicious variety (Mukhtar *et al.*, 2010) that may provide more nutrients to pathogen and resulted in highest lesion diameter of pathogenic growth.

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