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COMPARATIVE EFFECTS OF POSTHARVEST MITIGATION TREATMENTS ON MYCOTOXINS PRODUCTION POTENTIAL OF ASPERGILLUS PARASITICUS IN MANGO (*MANGIFERA INDICA* L.) FRUIT

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A B S T R A C T

This study focused on comparative effects of post-harvest low-to-high γ-irradiation doses (0.5–1.5 kGy), UV-C (30-60 min) and hot water treatments (55 ˚C for 5 min) upon spoilage of mango (*Mangifera indica* L.) fruit cv. *Kala Chunsa* by fungal infections. Thirteen fungal species belonging to five genera were isolated from diseased mango fruits surface. *Aspergillus* species were the most predominant followed by *Penicillium* and *Fusarium*. The aflatoxins AFB_1, AFB_2, AFG_1 and AFG_2 produced by *A. parasiticus* isolates on yeast extract sucrose (YES) medium for un-treated mango fruits was found 1.789, 0.52, 0.296 and 0.85 ppb, respectively. The increase in post-harvest γ-irradiation dose showed a high suppression of aflatoxins production ($p \leq 0.05$). Fruits treated with UV-C for 30 min showed higher aflatoxins production than fruits treated for 60 min ($p \leq 0.05$). The treatment of fruits with hot water also showed remarkable reduction in aflatoxins levels with respect to UV-C doses and control ($p \leq 0.05$). The results of this study demonstrated that treatment of mango fruit surface with post-harvest γ-irradiation dose (1.5 kGy / 2 hour and 15 minutes) or hot water (55 ˚C for 5 min) can inhibit the production of toxigenic aflatoxins. In conclusion, irradiation of fresh fruits such as mango can be effectively used on a practical scale to increase the international trade.

Keywords: Gamma Irradiations, hot water, mango fruit, mycotoxins, UV-C.

INTRODUCTION

Mango (*Mangifera indica* L.) is the most important fruit grown in tropical and subtropical region of the world. “The king of the fruits”, mango fruit has gained the popularity in the world due to its unique flavor and delicious taste. Mango fruit contains aminoacids, carbohydrates, fatty acids, minerals, organic acids, proteins and vitamins. It has an attractive fragrance and diuretic values with high health promoting qualities (Fulgoni *et al*., 2012).

The problem of agricultural commodities such as mango fruit with aflatoxigenic fungi has received great deal of attention during the last two decades. The fungal species such as *Aspergillus flavus* and *Aspergillus parasiticus* (Raper and Fennell, 1965) contaminate mango fruits at different points of the food chain, such as preharvest, processing, transportation or storage by producing aflatoxins (Wilson and Payne, 1994). The colonies of these fungi grow rapidly and the diameter of the colony reaches 6-7 cm within 7-10 days. The color of the colonies varies from yellow green to olive and dark green. The shape of colonies is with radial wrinkles (Yabe and Nakajima, 2004). Aflatoxins are the most potent natural carcinogens, mutagens and teratogens (Massey *et al*., 1995). The toxigenic species of fungi particularly *A. parasiticus* may reduce nutrient contents of the food and produce mycotoxins that could cause serious health hazards to humans (Pitt, 2000). *A. parasiticus* has different toxigenic profiles as it produces aflatoxin B_1, B_2, G_1, G_2 and versicolorin Aflatoxin A, B_1, B_2, G_1 and G_2 are chemical derivatives of difurancoumarin (IARC, 2001). In response to the current public health concerns with the microbiological safety of fresh and fresh-cut produce, researchers have investigated the efficiency of a number of very different methods for postharvest control of fungal decay of fruits. However, there are some limitations to the techniques that can be

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used such as the presence of chemical residues in the products and heat injury to mango fruits due to inappropriate temperature of conventional hot water treatment (Jitareerat *et al*., 2005). It has been reported that exposure to low UV-C doses (190–280 nm wavelength) delayed ripening and senescence of mango fruits. UV-C treatments also have direct effects on both vegetative and sporulating fungi (Marquenie *et al*., 2003).

During the last decade, science has provided the information about irradiation technology that can help to meet quarantine requirements (Fan, 2012). Food irradiation is a cold pasteurization technology for reducing post-harvest losses, extending shelf life of perishable commodities, improvement of hygienic quality of foods, and inactivation of food borne pathogens and parasites (Thayer *et al*., 1996). The irradiation technology may directly harm the genetic material of the living cell, leading to mutagenesis and eventually to cell death of microorganisms (Smith and Pillai, 2004). This paper reports a study aimed at determining the isolation and identification of different species of fungi from diseased mango fruit cv. *Kala Chunsa* and production potential of isolated *Aspergillus* species to produce aflatoxins.

MATERIALS AND METHODS

Aflatoxin standards for the experiments were purchased from Sigma chemical company, USA. A stock standard solution of aflatoxin at 10000 µg/mL in methanol was prepared and kept wrapped in aluminum foil at -20 ˚C. Aflatoxin working standard solutions were prepared by dilution in the same solvent and stored in glass stoppered tubes at 0 ˚C. All solvents used for the experiments (methanol, acetonitrile, n-hexane) were HPLC grade.

Preparation and Processing of Mango Fruits: Fresh and mature green mango fruits (cv. *Kala Chunsa*) were purchased (at the same day of harvest) from the farm located in Multan a city of Pakistan. After proper cleaning, mango fruits were transported at 11 ˚C in reefer container to Post Harvest Research Center. The mango fruits were divided into different groups randomly for application of the post-harvest treatments and were transferred to cold storage room at 11 ˚C. The γ-irradiations, UV-C and hot water treatments plan for fungal growth inhibition effect on mango fruits surface is presented in Table 1. Mango fruits were irradiated with 0.5, 1, or 1.5 kGy of ^{137}Cs-generated γ-rays using a Gamma-cell Elan 3000 (Elitemodel D, Nordion International, Inc., Ottawa, Canada). The fruits belong to ultraviolet treatment were exposed to radiation (UV-C< 280nm) for 30 and 60 minutes period. Diseased mango samples (n = 63) comprising of 3 mango fruits for each replicate of treatment were collected. These samples were kept in polythene bags separately and stored in a refrigerator prior to inoculation on to culture media.

Isolation and Identification of Fungi: Diseased Mango samples were initially surface sterilized with 70% ethyle alcohl washed twice with distilled water and placed on blotter paper to remove excess moisture and then inoculated on potato dextrose agar (Samson *et al*., 2004) and sub-cultured on Czapek dox agar (Pitt and Hocking, 1997) and yeast extract sucrose (YES) agar medium. Inoculated plates were incubated at 28 ˚C for 10 days. After the incubation period outgoing colonies were picked and transferred to plates containing different medium for purification. The isolated fungi were examined for colonial morphology and slide culture characters on different culture media (Klich and Pitt, 1988; Singh, *et al*., 1991). The isolation frequency (Fr) of the species was calculated according to Gonzalez *et al*. (1995) as follow:

$$Fr\ (\%) = \frac{No.\ of\ samples\ with\ species}{Total\ number\ of\ samples} \times 100$$

Aflatoxin Analysis: *Aspergillus* flavus was inoculated on YES medium at three equidistant points on a Petri plate and incubated at 27 ˚C for 10 days in dark. Fungal cultures were extracted by micro-scale extraction (Smedsgaord, 1997) with a modification that a total of 18 plugs (6 mm diameter) were cut from each plate in equal number from the middle, rim and areas between the colonies. These plugs were transferred to a 10 mL glass screw-capped vial containing 3 mL solvent mixture comprising of methanol: dichloromethane: ethyl acetate (1:2:3) containing 1% ($^{v}/_{v}$) formic acid and were extracted ultrasonically for 60 minutes. An aliquot of 0.5 mL of the extract was shifted to a glass vial and evaporated to dryness under a gentle stream of nitrogen. The evaporated residues of 0.5 mL extract were re-dissolved ultrasonically for 10 minutes in 400 µL methanol containing 0.6% ($^{v}/_{v}$) formic acid, 0.02 % ($^{v}/_{v}$) hydrochloric acid and 2.5% ($^{v}/_{v}$) deionized water. For aflatoxin analysis part of the extract was derivatized (Saleemi *et al*., 2010). Sample extract was dried under liquid nitrogen and 200 µL n-hexane was added and vortexed for 30 seconds to suspend the afltoxins.

Trifloro acetic acid (TFA) (50 µL) was added and vortexed again for 30 seconds to ensure homogenization of the mixture which was allowed to stand for 5 minutes. Water acetonitrile mixture 1.95 mL (9:1) was added and vortexed for 30 seconds again. The samples were allowed to stand for the separation of two layers, the lower layer was taken and filtered by using 0.45 micron syringe filter. A sample of the final filtrate (20 µL) was injected to HPLC system for quantification of aflatoxin. The aflatoxins were identified with reference to retention time of standards and by spiking the samples with standards. The standard curves were developed using concentration versus peak area for quantification of aflatoxins. Analysis was performed using LC-Shimadzu software run on the high pressure liquid chromatography system (Prominence™, Shimadzu® equipped with florescent detector RF-10 AXL® Shimadzu) by using C-18 column, Mediterranean Sea 18® 5µm 25 cm x 0.46 (Teknokroma, Spain). Mobile phase consisted of a mixture of acetonitrile methanol: water (22.5:22.5:55) with the flow rate of 1.0 mL/min, 30 ˚C. The emission and excitation wavelengths were 360 nm and 440 nm, respectively.

Statistical Analysis: The results were analyzed statistically by using Complete Randomized Design (CRD) using SPSS package (Steel and Torrie, 1980) and means were separated by applying LSD test.

RESULTS AND DISCUSSION

In the present study, three main treatments were analyzed in detail for their effects on fungal growth and aflatoxins production on the surface of mango fruit cv. *Kala Chunsa*. They include γ-irradiation, UV-C and hot water treatments. Thirteen fungal species belonging to five genera were isolated from the surface of diseased mango fruits. *Aspergillus* species were the most predominant followed by *Penicillium* (Samson and Pitt and *Fusarium* link Karst, Tousson and Nelson) Wollen weber and ReinKing. The fungal species isolated from mango fruits were *Aspergillus flavus (*Johann Heinrich Friedrich Link*, Aspergillus niger* Burnie, Mathews)*, Aspergillus fumigatus* (Berg Garbar *, Aspergillus oryzae* Wirsel) *Aspergillus terreus* (Thom)*, Aspergillus parasiticus (*Klich and Pitt, 1988). *Pencillium chrysogenum* (Thom)*, Pencillium brevicompactum* (Dierckx)*, Pencillium oxalicum* (Currie and Thom)*, Pencillium verrucosum, Cladosporium cladosporoides* (Fresen) G.A.de Vries)*, Fusarium oxysporum* (Synder and Hansen) and *Scopulariopsis brevicaulis* (Bainier) and their percent isolation frequency (Fr) was 16.52, 20.58, 0.87, 6.67, 7.54, 1.45, 6.1, 5.22, 3.77, 2.61, 14.78, 6.1 and 7.83, respectively (Table 1). (El-Samahy *et al*., 2000) also found fourteen fungal species belonging to five genera, *Aspergillus*, *Cladosporium*, *Penicillium*, *Fusarium* and *Scoplariopsis* on the surface of mango fruits.

Table. 1. Frequency distribution of isolated fungi.

Fungi isolated	No. of isolates	Fr %
Aspergillus flavus	57	16.52%
Aspergullus niger	71	20.58%
Aspergullus fumigatus	03	0.87%
Aspergullus oryzae	23	6.67%
Aspergullus terreus	26	7.54%
Aspergullus parasiticus	05	1.45%
Pencillium chrysogenum	21	6.1%
Pencillium brevicompactum	18	5.22%
Pencillium oxalicum	13	3.77%
Pencillium verrucosum	09	2.61%
Cladosporium cladosporoides	51	14.78%
Fusarium oxysporum	21	6.1%
Scopulariopsis brevicaulis	27	7.83%
Total	345	

Aflatoxins production potential of *Aspergillus parasiticus* isolated from diseased mango fruits subjected to different treatments has been described in Table 2. The maximum percentage of aflatoxins production was observed for *A. parasiticus* isolated from un-treated mango fruits surface. The aflatoxins AFB_1, AFB_2, AFG_1 and AFG_2 producing potential of toxigenic *A. parasiticus* isolates on YES medium for un-treated mango fruits was

found 1.789, 0.52, 0.296 and 0.85 ppb, respectively. The aflatoxins production potential of *A. parasiticus* isolated from diseased mango fruits subjected to different post-harvest γ-irradiations was dose dependent. In this study, it was found that increase of post-harvest γ-irradiation dose showed a high suppression of aflatoxins production ($p \leq 0.05$). The high-dose post-harvest γ-irradiation (1.5 kGy/2 hour and 15 minutes) showed the most effective treatment for suppression of fungal growth and aflatoxins production. Results showed that γ-irradiation at any dose significantly decreased aflatoxins production compared to control ($p \leq 0.05$). Fungal species vary widely in their resistance to irradiation, multicellular or bicellular spores are more resistant to gamma radiation than unicellular spores. Irradiation destroys chromosomal DNA of cells due to which microorganisms cannot survive (Bintsis *et al.*, 2002). The treatments of mango fruits at low-dose post-harvest γ-irradiation showed the slow growth of fungi may be due to some cells which were a little damaged, and cells were able to repair themselves later (Farkas, 2001). The presence of water content in the fungus cell, vegetative or reproductive form and number or density of mycelial cells in the inoculum exposed to radiation may affect the radiation dose required for the inactivation and further aflatoxins producing ability (Barkai, 1992; 2001).

The UV-C treated mango fruits were also found strongly correlated with the fungal growth and aflatoxins production. Fruits treated with UV-C for 30 min presented higher aflatoxins production potential than fruits treated for 60 min ($p \leq 0.05$). Gonzalez-Aguilar *et al*., (2007) recorded the overall appearance in relation with fungal decay for UV-C treated mango fruits and found lower decay for 10 min treatment than fruits treated for 5 min. The treatment of fruits with hot water (55 ˚C for 5 min) also showed significant reduction in aflatoxin levels with respect to UV-C doses and control ($p \leq 0.05$). The treatment of mangoes with post-harvest low-to-high γ-irradiation doses or UV-C and HW showed that there is very low concentration of aflatoxin production and is found in the range of permissible level as recommended by FAO/WHO (1987).

Table 2. Percentage of aflatoxins production potential of isolated *Aspergillus parasiticus* from surface of mangoes as a result of different treatments.

	Aflatoxin concentration (ppb)			
Treatments	AFB_1	AFB_2	AFG_1	AFG_2
Control	1.789	0.52	0.296	0.85
0.5 KGy / 45 min.	1.714	0.286	0.056	0.124
1 KGy / 1 h, 30 min	0.721	ND	ND	0.115
1.5 KGy / 2 h, 15 min.	ND	ND	ND	ND
UV-C / 30 min.	1.757	0.298	0.094	0.51
UV-C / 1 h	0.408	ND	ND	ND
Hot water 55 ºC / 5 min.	ND	ND	ND	ND

CONCLUSION

The results of this study demonstrated that post-harvest γ-irradiation dose (1.5 kGy / 2 hour and 15 minutes) and hot water treatment (55 ˚C for 5 min) effectively inhibited the production of toxigenic aflatoxins on mango fruit surface against other mitigation treatments. Data obtained from analysis suggest that gamma irradiation 1.5 kGy or treatment of mangoes with hot water at 55 ˚C for 5 min can be recommended to reduce the concentration of aflatoxins and microbial loads for phytosanitary measures.

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