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Effect of Gamma Irradiation, UV-Irradiation and Hot Water Treatment on Fungal Growth and Aflatoxin in Mango Fruits (*Mangifera indica* L.)

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Abstract: The comparative effect of gamma irradiation doses (0.5 kGy), UV-C-30 min and hot water 55°C-10 min on fungal load and aflatoxin production was evaluated on mango (black chaunsa). Thirteen fungal species were isolated from the surface of mango fruits (*Mangifera Indica* L.), among these the frequency of *Aspergilus niger*, *Aspergillus flavus* and *Cladosporium cladosporoides* was higher. Fungal load can be controlled best by using gamma rays at 0.5 KGy dose level followed by UV-C irradiation treatment while maximum load was found in control group. It appeared that non thermal techniques (gamma irradiation and UV-C) are comparatively better over control and conventionally used hot water treatment. Regarding the production of aflatoxins by these isolates, gamma irradiation treated samples showed less production of aflatoxin. Based on these results gamma irradiation and UV-C treatment possess the potential to replace conventionally used hot water treatment in mango.

Key words: Gamma irradiations, hot water, UV-C, microbial load, fungus frequency, sensory attributes

INTRODUCTION

"Mango is coined as "The king of fruits" mango and is largely grown in tropical and subtropical region of the world. It has gained the popularity in the world due to its excellent flavor, attractive fragrance, beautiful color, delicious taste. Asia is the main producer with 76.9% of the total world production of mangoes. Among countries, Pakistan is at 5th number in mango production, producing 938000 tons per year with a share of 7.6% in the world market (Ravi *et al*., 2011). Mango is a short seasoned fruit and being highly perishable does not withstand even in cold storage (Hussain *et al*., 2003).

A lot of problems related to postharvest life of mango are associated with microbial and fungal deterioration of fruit. These problem received a great deal of attention during the last two decades. The fungal species such as *Aspergillus flavus* and *Aspergillus parasiticus* contaminate mango fruits at different points of the food chain, such as preharvest, transportation, storage or processing (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 green and dark green. The shape of colonies is smooth or radial wrinkles (Yabe and Nakajima, 2004). These fungi also have the ability to produce Aflatoxins, which are the most potent natural carcinogens, mutagens and teratogens (Massey *et al*., 1995). Conventionally, Hot water treatment is used as a tool to prevent postharvest deterioration of the mango fruit. There is a big scope of other techniques to retard the contamination of fungi on these fruits these may include use of gamma irradiation and use of ultra violet radiations. Science has provided information about irradiation technology that can help to meet hygienic requirements in fruits and vegetables (Fan, 2012). If used judiciously, it can reduce post harvest losses, increase shelf life, improve hygienic quality and inactivation pathogens of food (Thayer *et al*., 1996). This study aimed to observe the effect of gamma irradiation, UV-irradiation and hot water treatment on fungal growth of mango fruit cv. *blackchunsa* during storage. The outcome of the research will benefits the food industry as well as to the retail markets.

MATERIALS AND METHODS

Preparation and processing of mango fruits: Fresh and mature green mango fruits (*Black Chuansa*) were purchased (at the same day of harvest) from the farm located in Multan city of Pakistan. After proper cleaning, mango fruits were transported at 11±1°C in refrigerated container to Post Harvest Research Center, Ayub Agriculture Research Institute, Faisalabad, Pakistan. 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±1°C. The γ-irradiation (treated at NIAB, Faisalabad), UV-C and Hot water treatments (treated at Ayub Agriculture Research Institute, Faisalabad) plan is described in Table 1. Mango fruits were irradiated with 0.5 kGy of ^{137}Cs-generated γ-rays using a Gamma-cell Elan 3000

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Table 1: Treatments applied to mango fruits

Trt. Rep.	Name of Trt. Appl.	Dose of Treatment
T_0	Control	-
T_1	Gamma rays	0.5 KGy/50 mn
T_2	Ultraviolet	UV-C for 30 min
T_3	Hot water	Hot water 55°C for 5 min

Trt. Rep.: Treatment representation, Name of Trt. Appl.: Name of treatment applied

(Elitemodel D, Nordion International, Inc., Ottawa, Canada). The fruits belong to ultraviolet group were exposed to radiations (UV-C<280 nm) for 30 min period. Mango fruits were subjected to hot water treatment in cotton bags at 55°C for 10 min and immediately cooled by dipping in cold water at 20°C and air dried. The samples were kept in cardboard packs that were stored at 25±5°C and relative humidity 40-50%. The data were recorded after 25 days.

Isolation and identification of fungi: Mango samples were initially inoculated on potato dextrose agar (Samson *et al*., 2004). These samples were then sub cultured on Czapek dox agar (Pit and Hocking, 1997) and yeast extract sucrose agar. Inoculated plates were incubated at 28°C for 7-10 days. After the incubation period, the isolated fungi were examined for colonial morphology and slide culture characters on different culture media (Singh *et al*.,1991; Klich and Pitt, 1988). The isolation frequency (Fr) of the species were calculated according to Gonzalez *et al*. (1995) as follow:

$$\text{Frequency}(\%) = \frac{\text{No. of samples with a species or genus}}{\text{Total no. of samples}} \times 100$$

Aflatoxin analysis: *Aspergillus* isolate 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 (Smedsgaard, 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. A 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 vertex for 30 sec to resuspend the afltoxins. 50 µL Trifloro acetic acid (TFA) was added and vertexes again for 30 sec to ensure homogenize the mixture and allow standing for 5 min. Add 1.95 mL water acetonitrile (9:1) and vertex for 30 sec again. The samples were allowed to stand for the separation of two layers, lower layer was taken and filtrate by using 0.45 micron syringe filter. 20 µL of the final filtrate 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, Mediterancan Sea 18® 5 µm 25 cm x 0.46 (Teknokroma, Spain). Mobile phase consisted of a mixture of acetoritrile: 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 and 440 nm, respectively. The results were analyzed statistically by using Complete Randomized Design (CRD) as recommended by Steel and Torrie (1980).

RESULTS AND DISCUSSION

All treated samples reduced variably the prevalence of fungal isolates in mango fruit (var. black chaunsa). Fruits are often vulnerable to attack of various types of fungi during storage and their growth conditions may be variable but there are acceptable evidences their growth may appear after 2-3 weeks depending upon storage conditions. In tropical countries most producers either stored the mangoes fruits as such after harvesting or provide a hot water treatment before storage. This research indicated comparatively a higher number of isolates in control samples (without any treatment). The total number of fungal isolates were 441, *Aspergilus niger*, *Aspergillus flavus* and *cladosporium cladosporoides* were the dominant (Fig. 1). The frequency of occurrence of *Aspergilus niger*, *Aspergillus flavus* and *cladosporium cladosporoides* in control samples were 20.63%, 17.23 and 14.29%, respectively. These three types of isolates collectively constitutes more than half of all the fungal isolates in control samples (Fig. 2). Other minor fungal isolates of importance were *scopulariopsis brevicaulis*, *fusarium oxysporum*, *aspergillus oryzae*, *aspergillus parasiticus*, *aspergillus terreus*.

Fungi varied in their resistance to different treatments. Number of fungi isolates from mangoes subjected to gamma irradiation treatment have been shown in Fig. 3. The T_1 (0.5 KGy gamma irradiation) was the most effective treatment in controlling the fungal population on mangoes. The total isolates in T_2 treated samples were 156 as against 441 in control samples. This corresponds to about 64% decrease in fungal isolates by using mild treatment of gamma irradiation (0.5 KGy). The frequency of occurrence for *Aspergilus niger* and

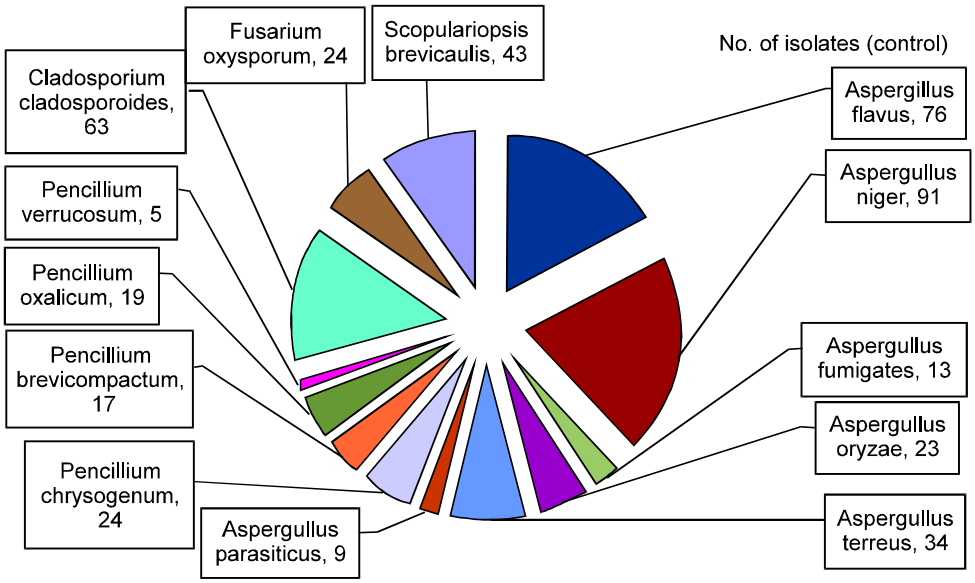

Fig. 1: Number of fungal isolates (Control samples)

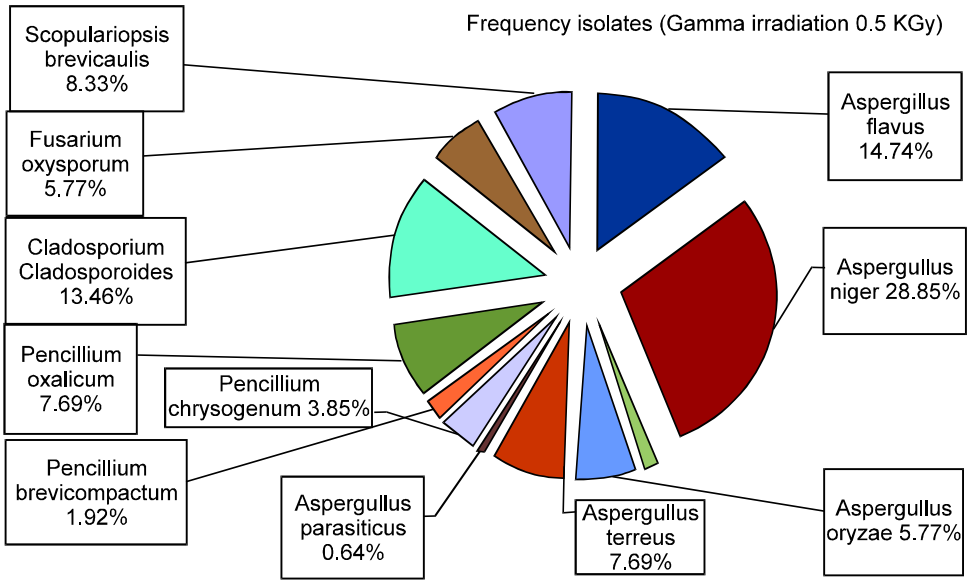

Fig. 2: Frequency of Isolate (Control)

Aspergillus flavus was highest, rest of fungus appeared at less than 10% (Fig. 4). While comparing fungal isolates in gamma irradiation treated samples over control samples, all fungal isolates decreased to an appreciable level over control samples. It is also notable that *Pencillium verrucosum* was absolutely absent in gamma irradiated samples. Significant decrease in *Aspergilus niger, Aspergillus flavus* and *cladosporium cladosporoides* was recorded that constitutes more than half of the isolates in control samples. This high reduction in fungal growth due to mild dose of gamma radiation could be attributed to the direct and indirect effects of radiation on genetic material of fungus. Mild doses of irradiation usually are effective in controlling unicellular or bicellular spores of fungus. Higher doses are required to have a killing action on multicellular fungus (Beraha, 1964; Sommer *et al*., 1964).

In this study the effect of another treatment UV-C was evaluated on fruit. It's a non conventional treatment and often used for treatment of packaging material only. In this study this treatment showed a positive effect on controlling fungal population in mango fruit. But when we compare it against gamma irradiation, it appeared to be less effective. Comparing UV-C treated samples with

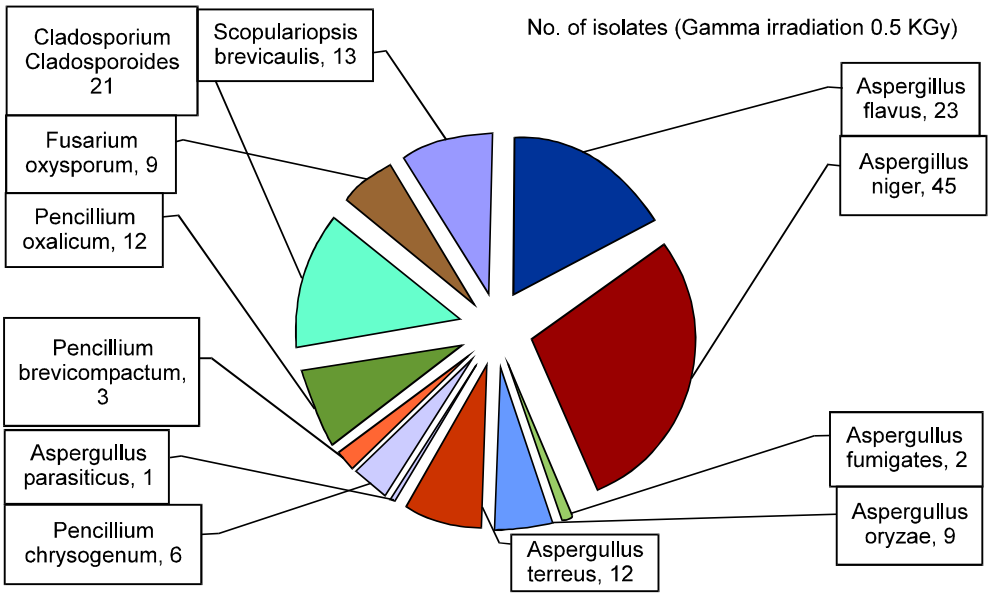

Fig. 3: Number of fungal isolates (Gamma Irradiated samples)

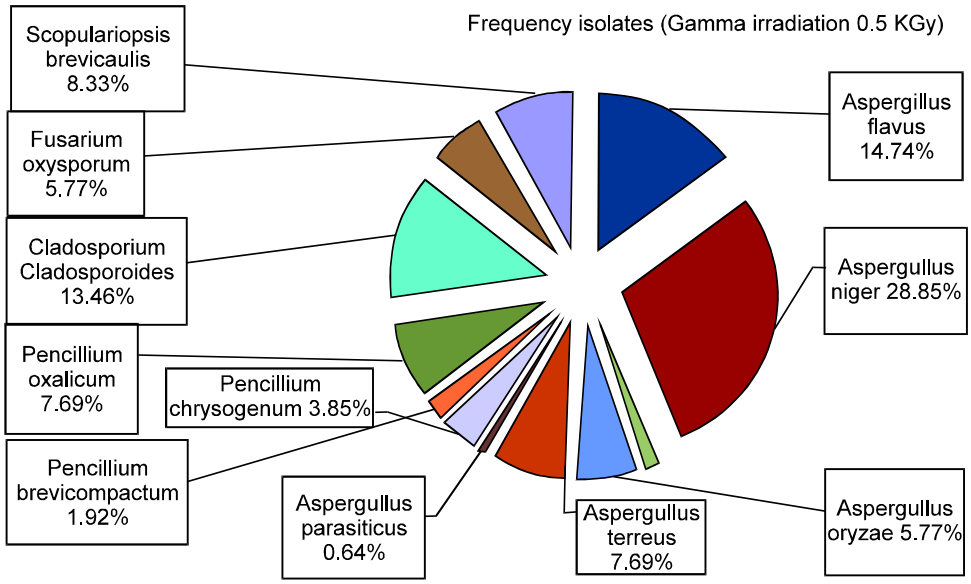

Fig. 4: Frequency of Isolate (Gamma Irradiation treatment)

control contaminated with 441 isolates, the UV-C treatment for 30 minutes restrict the total fungal species to a value of 282 isolates with highest number of isolates of *Asergillus niger* (Fig. 5). This corresponds to about 36 % reduction in fungal isolate over control samples. The frequency of occurrence for *Aspergilus niger*, *Aspergillus flavus* and *Cladosporium cladosporoides* was the highest with 20.57, 15.25% and 11.35, respectively (Fig. 6). Rest of all fungus appeared at less than 10 %. These results conclude that UV-C has a positive effect in controlling fungal growth but is less effective as compared to gamma irradiation and can be used selectively if facility of gamma irradiation is not available.

Hot water treatment is a conventional treatment renowned for most of the mango farmers. The data indicated that sample treated with this treatment also reduced the number of isolates in mango fruits but when we calculate the (%) decrease in fungus isolate over control samples, a small decrease of about 12% is evident in number of fungus isolates in this treatment. A grand total of 288 isolates were observed in this

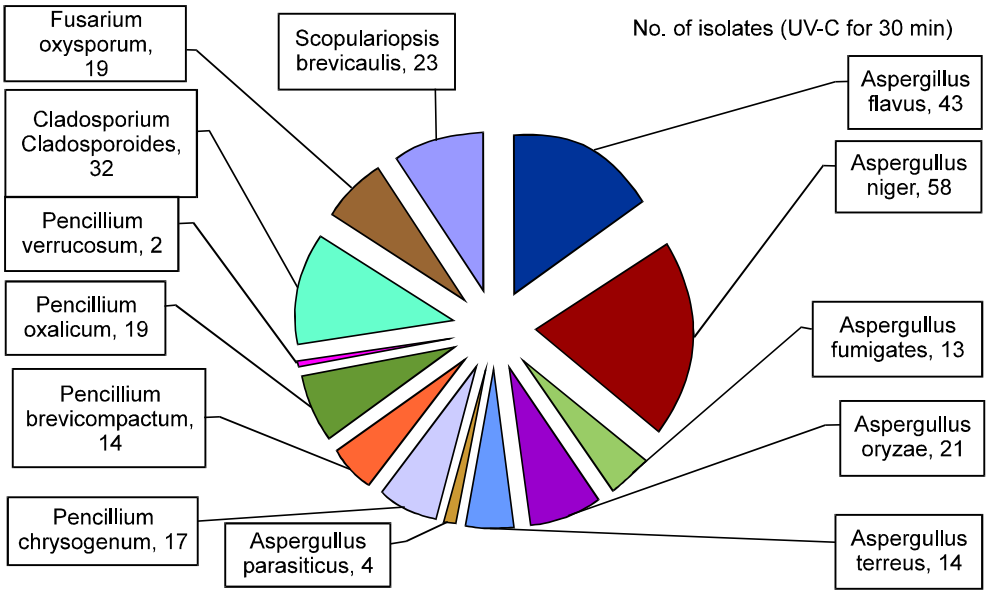

Fig. 5: Number of fungal isolates (UV-C treated samples)

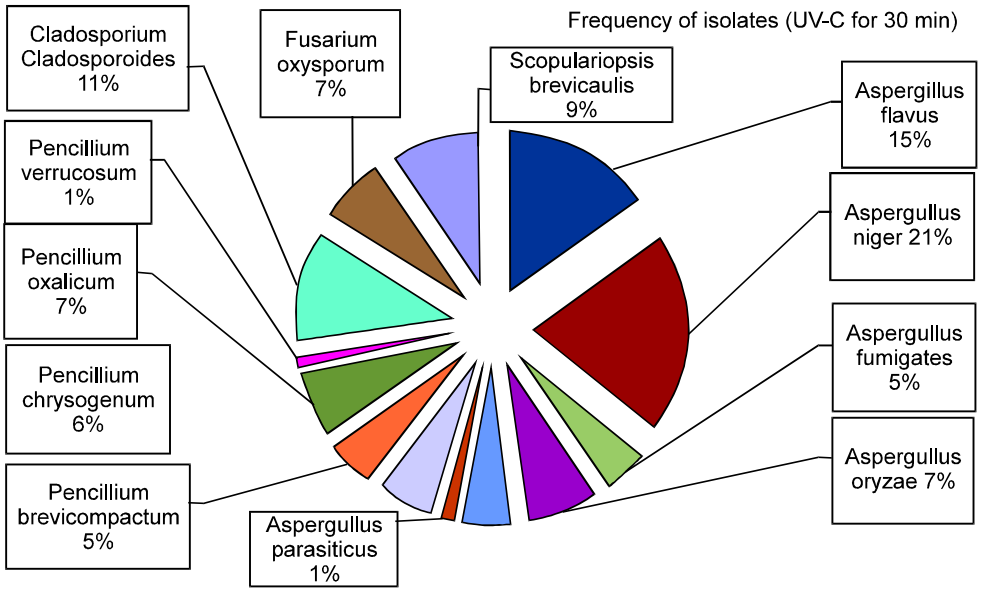

Fig. 6: Frequency of Isolate (UV-C treatment)

treatment out of these *Aspergilus niger*, *Aspergillus flavus* and *Cladosporium cladosporoides* type of fungus dominate the other fungus (Fig. 7). Frequency of occurrence for *Aspergilus niger, Aspergillus flavus* and *Cladosporium cladosporoides* was 22.94, 17.78 and 14.43%, respectively (Fig. 8).

The maximum percentage of aflatoxins production was observed for *A. parasiticus* isolated from treated or untreated mango fruits surface. Gamma irradiated samples showed less occurrence of *A. parasiticus* as compared to other treated or untreated samples. 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, gamma irradiated, UV-C treated and hot water treatment fruits was found 1.789, 0.22, 0.596 and 0.95 ppb, respectively. The ã-irradiation (0.5 kGy) appeared as the most effective treatment for suppression of fungal growth and aflatoxins production. Irradiation and UV-C destroys chromosomal DNA of cells due to which microorganisms can not survive (Bintsis *et al*., 2002). The treatments of mango fruits at low-dose post-harvest ã-irradiation cause disruption of cell wall and most of the microorganisms are unable to repair themselves later (Farkas, 2001). The dose

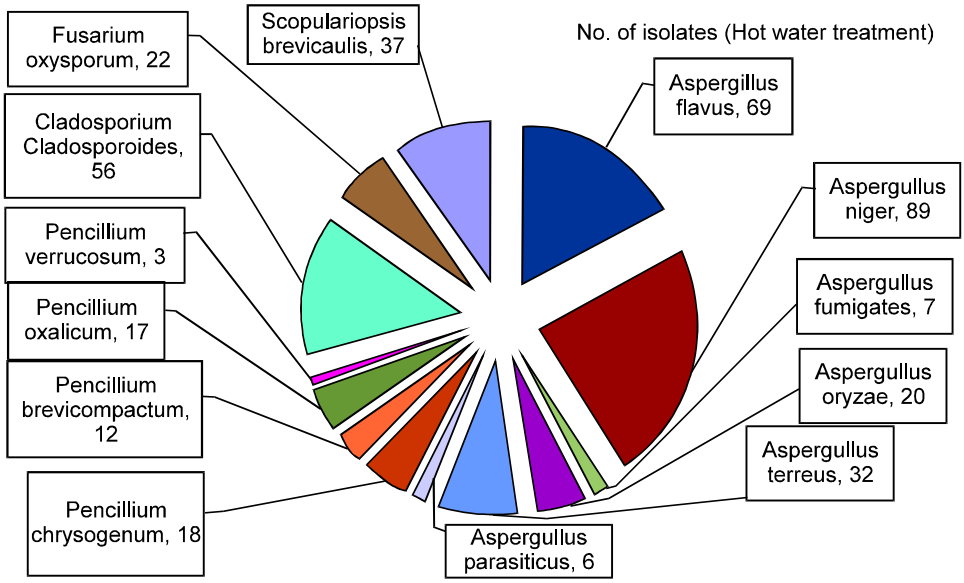

Fig. 7: Number of fungal isolates (Hot water treated samples)

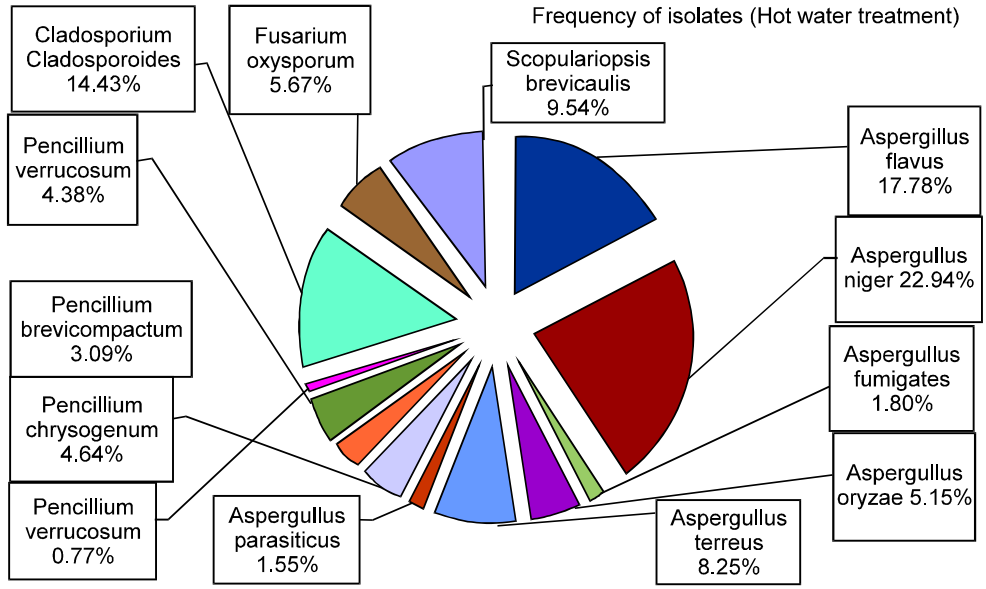

Fig. 8: Frequency of Isolate (Hot water treatment)

dependence for killing of these microorganisms depends upon the presence of water content in the fungus cell, vegetative or reproductive form and number or density of mycelial cells. These factors also affect the radiation dose required for the inactivation and further aflatoxins producing ability of these fungus (Barkai-Golan, 1992, 2001). The UV-C treated mango fruits were also found strongly correlated with aflatoxins production. Gonzalez *et al.* (2007) recorded the overall appearance in relation with fungal decay for UV-C treated mango fruits and found lower decay for 5 min treatment than fruits treated for 10 min. The treatment of fruits with hot water (55°C for 5 min) also showed significant increase in aflatoxin levels with respect to UV-C, gamma irradiation and control ($p = 0.05$). The treatment of mangoes with post-harvest low γ-irradiation doses or UV-C and hot water treatment showed that the concentration of aflatoxin production is inevitable but luckily it is found in the range of permissible level as recommended by FAO (1987).

Conclusion: Nonthermal technologies used in this study, gamma irradiations UV-C have a better impact in controlling fungus growth. There is a great potential to

use these preservation techniques over conventional hot water treatment for mango fruits. These non thermal techniques, especially gamma irradiation is much effective in reducing aflatoxin level on contaminated fruits. This will eliminate the use of elevated temperatures during processing and will be helpful for keeping sensory and nutritional properties of fruit intact. Such approach of adopting minimal processed food will have more desirability among the consumers.

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