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USE OF Co^{60} GAMMA IRRADIATION AGAINST SOME POSTHARVEST DECAY PATHOGENS OF CITRUS FRUIT IN TURKEY*

T. MAHMOOD†

Department of Plant Pathology, Faculty of Agriculture, Ege University, Izmir, Turkey

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Abstract. Investigations were carried out *in vivo* and *in vitro*, on some decay pathogens, to determine the possibility of using Co^{60} gamma irradiation, to extend the shelf and storage life of citrus fruit. Tests *in vivo* showed that a dose of 200–300 Krad was effective for decay control incited by *Penicillium italicum*, *P. digitatum*, *Phytophthora citrophthora*, *P. parasitica*, and *Geotrichum candidum*. The problem of organoleptic changes was mostly encountered when exploiting the above determined dosages in case the quality of the fruit was poor, whereas in good quality fruit the dosages were efficient and remarkably increased the storage and shelf life. However, a low dose of 100–200 Krads has been suggested. In *in vitro* tests the germination of the spores of decay pathogens and their rates were correspondingly influenced with the increasing amount of gamma dose delivered. A higher dose caused a depletion in vitamin C content of citrus fruit.

Citrus is an important fruit in Turkey and a good item of export for its neighbouring European countries. Its annual planting area and the total production is expanding.[16] With the increasing emphasis on export, the present investigation was carried out to determine the feasibility of using Co^{60} gamma rays as a therapeutic mean of pasteurization against some decay pathogens to prolong the shelf and storage life of citrus fruit. The penetrable ability of the ionizing radiation into the host tissue to inactivate the fungus-colonies after infection, provides an efficient means as fungicidal treatment and supersedes over the use of chemicals which rarely penetrate extensively into the host's living tissues. It has been reported that some workers [9,10,12,19,20] used 2,4-D and 2,4,5-T against citrus decay pathogens as packing-house experiments in California, and achieved some success to increase the shelf and storage life of lemons and oranges. In the recent years, the use of Co^{60} gamma rays against citrus decay pathogens has been investigated by many workers.[1,3,4,5,6,7,8,14,17,18] In the above reported investigations it has been found that a low gamma dose (100–200 Krads) irradiation may prolong the storage life of lemons and oranges. A permanent halt in decay was only achieved by inactivating every fungus cells harbouring in a lesion.

In the present investigations, some postharvest decay pathogens namely *P. italicum* Wehmer, *P. digitatum* Sacc., *P. citrophthora* (R.E. Smith and E.H. Smith) Leonian, and *P. parasitica* Dast., prevalent in Turkey on citrus fruit, were studied *in vivo* and *in vitro*, using Co^{60} gamma irradiation. *Geotrichum candidum* Lk., is also prevalent in Turkey as a postharvest decay pathogen causing sour rot of lemon,[13] and the effect of Co^{60} gamma rays on this decay pathogen has already been reported.[14]

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†Post-Doctoral Research Fellow, Alexander von Humboldt-Stiftung from West Pakistan. Now at Institut fur Pflanzenkrankheiten der Universitat Bonn, Nussallee 9, West Germany.

Materials and Methods

To isolate citrus decay pathogens the diseased portions showing lesions were cut off and surface sterilized with 0.5% solution of sodium hypochlorite. They were plated on a potato dextrose agar (PDA) medium and good sporulating cultures in 250 ml flasks were maintained for various tests. For the isolation of *P. citrophthora* and *P. parasitica* the technique used by Zentmeyer [21] was followed. Lemons found to be infected were collected and severely rotted portions were cut off. They were dipped briefly in 70% alcohol, allowed to dry and plated on oatmeal or cornmeal agar. Later it was observed that in order to isolate *P. citrophthora* and *P. parasitica* surface sterilization was unnecessary. Therefore, the infected portions were only rinsed in sterile water 3–4 times and directly plated on PDA medium.

For the pathogenicity tests, fresh and good quality lemons, oranges and mandarines were procured from the market. Each time the fruits were used 5–10 days after picking. They were surface sterilized twice with 70% alcohol and allowed to dry. Conidial suspensions of *P. italicum* and *P. digitatum* were prepared in sterile water. Incisions approximately 2 mm depth were made on the prepared fruits. The incised site of the fruit was briefly dipped in the conidial suspension and the fruit was immediately wrapped in a polyethylene bag and sealed. The inoculated fruit in the bag was provided with a humid atmosphere by atomizing sterile water into the bag.

Each replicate consisted of one fruit in a bag. In the case of *P. citrophthora* and *P. parasitica* the mycelia are incapable of causing infection of the lemon fruit. Therefore, zoospores were used for the pathogenicity tests. The cultures were grown for about 10 days at 20–25°C in cornmeal or oatmeal agar or PDA medium and later the mycelium was covered with a 1–5% solution of KNO_3 and incubated at 25°C. After 4–6 days sporangial formation was visible and zoospores were produced. The lemon fruits were scratched slightly and put in the zoospore suspension. After 24–36 hr brown spots appeared on the dipped portion of the lemon.

For the tests *in vitro* and *in vivo* the irradiation of the cultures and artificially inoculated fruits, respectively, were carried out at the Nuclear Atomic Energy Commission, Istanbul. Varying doses of gamma radiation (100, 200, 300, 400 and 500 Krads) were used but for brown rot of lemon caused by *P. citrophthora* and *P. parasitica* low doses of 50, 100, 150, 200 and 250 Krads were used. The radiation flux of gamma rays consisted of 2 Krad/min and the amount of administered dose was determined by the period of exposure of materials. In *in vivo* tests, the rate of infection after irradiation was scored against a scale showing 0 for no infection and grades 1, 2, 3, 4 and 5 after 3, 5, 7, 9, and 11 days, respectively, after incubation in storage at 25°C, showing the amounts of infection based on the size of rotted lesions in the inoculated and control fruits.

Results

Studies in vivo. *P. italicum* is the causal organism of blue mould rot of oranges (Washington Navel and Mandarine). A total dose of 100 Krad at a dose rate of 120 Krad/hr gave little control of decay in artificially inoculated oranges. A dose of 200–300 Krad was very effective for decay control. Higher gamma doses (400–500 Krads) impeded the sporulation and further growth of the fungus in the lesion (Fig. 1). A dose of higher order impaired marked peel damage, appeared brown spots on the rind and softening of the texture (organoleptic changes). Although with a higher dose there was a partial halt of decay but new growth appeared from the edges of the lesions after 13–20 days following irradiation. At a dose of 200–300 Krad the storage life was increased by 40–50 days against 9–11 days in controls (unirradiated).

P. digitatum is the causal organism of green mould rot of lemons. A dose of 100 Krad was ineffective to control the decay like *P. italicum*. At a total dose of 300 Krad, there was an appreciable arrest in decay of inoculated lemons and the storage life was increased by 30–40 days stored at 25°C against 9–11 days in controls. At doses of 300–500 Krad, dark brown bands showing radiation-induced injury appeared on the rind of the lemon fruits (Fig. 2).

P. citrophthora and *P. parasitica*, the causal agents of brown rot of lemon. The decay pathogen is found to be radiosensitive and a dose of 150 Krad was very effective to control the decay process (Fig. 3) and prolonged the shelf and storage life by 40–50 days in contrast to 11 days in controls. At a dose of 150 Krad, no or very mild radiation-induced injury was noticeable. The occurrence and manifestation of radiation-induced injury varied with the type of the lemons used for irradiation. In poor quality lemons the injury was much pronounced whereas in better quality it was almost absent.

Studies in vitro. The citrus decay pathogens were studied *in vitro*, suspended in water and in PDA medium. Conidia from richly sporulating cultures were transferred to small bottles and irradiated. The gamma irradiated cultures were used to determine the effect of ionizing radiation on germination, colony-inducing potential, and growth rate of the conidia of decay pathogens. Higher doses (400–500 Krad) were required to prevent germination of conidia of *P. italicum* and *P. digitatum*, than required to inhibit colony-inducing potential. The length of the germ-tube from the irradiated conidia of *P. italicum* and *P. digitatum*, was inversely proportional to the amount of gamma dose delivered: a higher dose resulted in shortened and distorted germ-tube. A total dose of 200–300 Krad influenced and delayed the germination period of the conidia by 20–25 hr. The mycelium of *P. citrophthora* and *P. parasitica*, was radiosensitive and on exposure to gamma doses of 400–500 Krad, there was a remarkable effect on the germination. The Co^{60} gamma radiation also influenced the total growth rate of mycelium and spores of decay pathogens (Fig. 4). When water

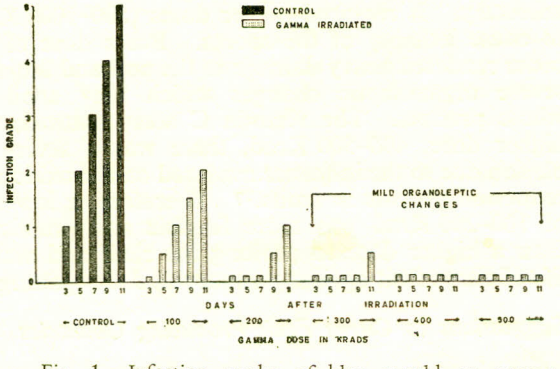

Fig. 1. Infection grades of blue mould on orange (Washington Navel) following irradiation with different doses of Co60.

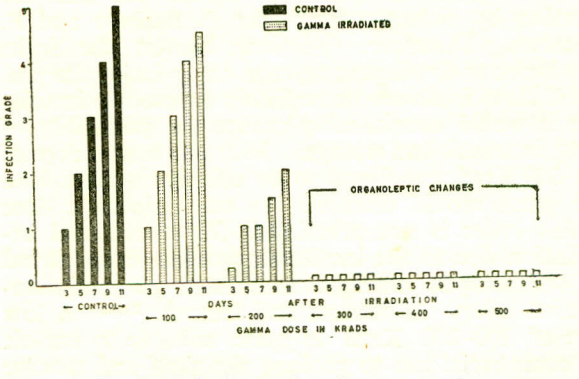

Fig. 2. Infection grades of green mould on lemon following irradiation.

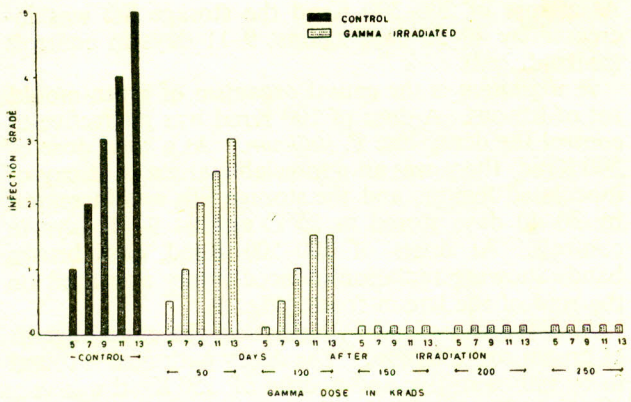

Fig. 3. Infection grades of brown rot on lemon following irradiation.

and PDA were compared as suspending media for the spores of *P. italicum* and *P. digitatum*, PDA was found to be more favourable for the survival. The basis of difference was not studied but it may be the result of a protective effect by PDA medium.

Organoleptic Changes. Freshly procured lemons and oranges (Washington Navel and Mandarine) following irradiation at the effective radiation doses (200–300 Krad) exhibited organoleptic changes of the varying degree. The most prominent features of such changes were damage of the peel, appearance of water-soaked and soft areas, and dark brown lesions on the rind showing radiation-induced injury. Lemons were less radioresistant in contrast to orange (Washington Navel). The development of organoleptic changes varied from fruit to fruit and in case of very good quality of the produce, the resistance was higher. The mandarine (*Citrus reticulata*) fruit was very radio-susceptible and following irradiation, manifested severe organoleptic changes, therefore, was unfit for radiopasteurization whereas Washington Navel orange (*C. sinensis*) could withstand radiation sterilization without any organoleptic change, and may be exploited for commercial use.

Vitamin C Assay. The influence of Co^{60} gamma radiation on the vitamin C content of the lemon and orange fruit was studied and is presented in Table 1.

Discussion

During the period of picking, packing and transit the fruits get injured and the injuries are sites for infection by airborne fungi like *P. italicum* and *P. digitatum*. Therefore, following harvest the fruits may harbour developing mycelia on or inside the peel and if these fruits are immediately exposed to irradiation it would considerably reduce the possibility of decay in transit and storage. In *in vivo* tests a dose of 200–300 Krad was found to be effective against blue and green mould diseases. In our opinion a dose of this order is not practicable for commercial exploitation due to the inevitable problem encountered by organoleptic changes manifested as radiation-induced injury to oranges and lemons. [2,14] A low dose of 100–200 Krad is quite suitable to exploit for commercial use to prolong the shelf and storage life of freshly picked fruits which may harbour fungi as developing mycelial cells. A low dose of this

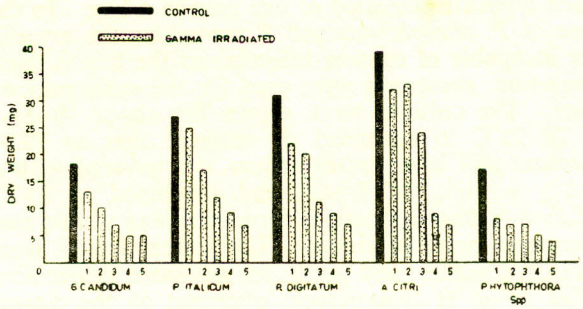

Fig. 4. The influence of different doses of irradiation on the growth rate of some citrus fruit decay pathogens (1,100; 2,200; 3,300; 4,400 and 5,500 Krads).

TABLE 1. CHANGE IN VITAMIN C CONTENT OF LEMON AND ORANGE (WASHINGTON NAVEL) FRUIT 15 DAYS AFTER Co^{60} GAMMA IRRADIATION WITH DIFFERENT DOSAGES.

Total dose (Krads)	Vitamin C (mg %)			
	Lemon		Orange	
	Irradiated	Control	Irradiated	Control
100	30·10	34·40	47·00	54·00
200	24·00	,,	48·00	,,
300	16·30	,,	29·00	,,
400	18·60	,,	21·00	,,
500	10·30	,,	18·00	,,

order as practically feasible has been reported by Beraha,[4,5,6,7,8] Rogachev,[17] and Baraki-Golan. [1,2] Brown rot disease of lemon was found to be radio-sensitive and the decay process was halted at a dose of 100–150 Krad. In *in virto* tests the suspending media showed an influence on the survival of spores. With PDA medium the survival was higher since ionizing reaction of gamma rays on the fungus spores was exclusively indirect whereas in water suspension there was a direct and indirect affect. In the same way it has been seen that a gamma dose required *in vitro* is not the same as required *in vivo* to inactivate the fungus. This variation is due to the host's influence[7] and probably the peel and juice provide a protection to fungus cells. In order to obtain a complete killing of the fungus spores and 100% half of the decay process in a lesion, a thorough inactivation of all fungal cells inhabiting the host tissue was necessary. It required higher doses (500–1000 Krad) to cause lethality of the spores. But a dose of this order rendered heavy damage to the peel and impaired severe organoleptic changes which may spoil the whole product. The vitamin C assay showed, at a higher dose, 400–500 Krad, there was a severe depletion due to the influence rendered on the oxidation–reduction process in fruits.[17] Therefore, a low dose of 100–200 Krad was more feasible and practicable than a higher dose to prolong the shelf and storage life of citrus fruit against postharvest decay pathogens.

Suggestions to Citrus Fruit Producing Countries

We would like to suggest to the citrus fruit producing countries that an investigation for determining

the possibility of increasing shelf-and-storage life of lemons and oranges by the use of Co^{60} gamma irradiation may be very useful in the interest of export expansion of the country. It would be useful to know the feasible gamma dose requirements of the citrus fruit produced in different environmental conditions in different countries of the world. And once when the feasible gamma dose for irradiation is established, it may be very economical and can be applied on a commercial scale. It may supersede all other laborious techniques of chemical treatments for the preservation of fruits for export or kept in storage for domestic consumption to avoid deterioration and decay by the microorganisms.

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