

Editorial

Advanced strategies for management of postharvest diseases of fruits and vegetables

Fruits and vegetables are protective food for human being by providing lifesaving vitamins and minerals. The produce of these crops perishable in nature and grown in developed and developing countries worldwide. On average, approximately 30–40% of the total produce is lost or thrown away at various stages of postharvest such as harvesting, sorting, cleaning, handling, and packing, transportation, storage, marketing, and processing. However, the postharvest losses in developed countries (5 - 35%) is slightly lower than in developing countries (20 -50%). Besides losses of produce in quantity, the quality of produce of fruits and vegetables is also influenced by diseases particularly nutritional losses, production of mycotoxin by fungi and reduction of market value. Postharvest losses of fruits and vegetables are far higher than those of cereal crops due to their higher size, more respiration rate and softening of the peel. The losses are caused by biotic and abiotic factors which include diseases, insects, rodents, thefts, mechanical damage, premature harvesting, harvesting of overmature crops, improper harvesting and storage facilities, lack of proper packaging and marketing system, and seasonal fluctuation of the products.

Many fungi and bacteria are associated to cause various diseases in fruits and vegetables during storage and transportation. However, the major postharvest diseases are caused by many fungal genera such as *Alternaria*, *Aspergillus*, *Botrytis*, *Colletotrichum*, *Cladosporium*, *Diplodia*, *Lasiodiplodia*, *Monilinia*, *Mucor*, *Penicillium*, *Phomopsis*, *Rhizopus*, and *Sclerotinia* and bacterial genera like *Bacillus*, *Clostridium*, *Erwinia*, *Dickeya*, *Janthinobacterium*, *Pectobacterium*, and *Pseudomonas*. Most of these organisms are weak pathogens in that they can only infect injured produce. Development of postharvest disease is affected by pathogen biology, growth stage, and handling of the produce at different stages. The most important environmental factors are temperature, relative humidity, carbon dioxide levels, nutrient availability, and physical damage of the produce. Physical damage is the primary cause of the losses which plays a significant role in postharvest spoilage and deterioration. Injury can be caused by weather, insects, birds, rodents, and farm implements before, and after the harvest of the produce. Injuries to the fruits usually occur when produce is dropped on to a hard surface, before, during or after packing, but injury is not usually immediately apparent. The damaged produce is attacked by these fungi and bacteria, resulting the spoilage of the produce.

Diagnosis of postharvest diseases of fruits and vegetables is mainly based on symptomology, morphological and biochemical characters of the pathogens. Recently, a biosensor technique has been developed which transform biological responses into detectable electrical signals. A few biosensors like antibodies, antimicrobial peptides, cells, organelles, microorganisms, enzymes, and nucleic acid are use dto monitor the pathogenic fungi and bacteria of the produce. The development of sensors based on phytochemicals or ripening ethylene genes is found effective to control the disease. A sensor composed of a metal catalyst and carbon nanotubes has been developed to monitor ethylene to determine the time of spoilage of the produce, based on the luminescent responses of the bacteria to changes in volatile compounds following contamination. Whole-cell bacterial biosensors can detect *Penicillium digitatum* in oranges. *Aspergillus niger* is being detected by using a colorimetric sensor containing AuNPs modified with the *A. niger* spore-binding peptide. For rapid monitoring of *Penicillium*, the electrochemical biosensors are used by immobilizing penicillinase enzyme using N-5-azido-2-nitrobenzoemideyl.

Generally, it is believed that good agricultural practices, safe harvesting, postharvest treatment, proper packaging and controlled storage conditions are necessary to minimize pathogen infection and extend shelf life of the produce. In recent past years, physical methods such as thermal treatments [hot water (50-60° C for 2-10 min), steam heat, and hot air] reduce surface contaminants by suppressing pathogen activity effectively. The heat treatment disrupts normal metabolism (ripening or senescence) by creating moderate and reversible stress. However, heat treatment has undesirable effects on nutritional quality like vitamin reduction, protein denaturation, and sensory properties. To minimize adverse effects of heat treatment, non-thermal techniques such as ionizing radiation (UV irradiation, Gamma irradiation, X-rays, cathode ray spectra) microwave, infrared, visible light, and ultrasound, cold plasma, and high-pressure processing pulse electric field radiation are used to treat the fresh fruits and vegetables. Irradiation induces resistance due to expression of pathogenicity-related proteins, the activity of antioxidant enzymes, the synthesis of phenolic compounds, and cell-wall strengthening. Irradiation delays the ripening of fruit but, care is needed with the dosage to avoid unwanted effects on quality. Cold plasma is an ionized gas containing partially ionized atoms and molecules with a roughly zero net electrical charge. It destroys fungal DNA and cell walls and allows the leakage of intracellular contents and causes rapid destruction of mycotoxins.

Use of low temperature is considered very important in controlling decay in several fruits and vegetables which slows down the growth of the pathogens as well as delays ripening process of the produce. Certain varieties of apple, pears, plums, peaches can thus be stored between 0 and -2°C while in case of tropical fruits cannot be stored below 10°C without expressing chilling injury symptoms. The pathogenic growth of most fungi, however, is completely stopped at temperature near 0°C but *Botrytis cinerea*, *Penicillium expansum* and *Cladosporium* can attack on apples and pears stored at slightly below 0°C. Respiration rate of fruit is reduced by decreasing oxygen level and increasing carbon dioxide level in storage atmosphere. This storage environment can be lethal for pathogen but if CO₂ or O₂ levels are provided for a long period, it may have negative impacts on the stored product. Strawberry is an exceptional example able to tolerate high levels of CO₂ usually 20–30% which has inhibitory effects for grey mold disease development. Development of postharvest disease is also influenced by the relative humidity of the storage house as water loss of the stored product is minimized by maintaining high relative humidity. However, it can promote the disease development if free moisture content is available in the storage containers. So, it is very important to choose proper relative humidity for storage house to prevent water loss and also minimize the disease development.

Besides traditional fungicides like benzimidazole, other fungicides offer alternatives which have lesser toxicological effects on mammals and impact on the environment. Generally recognized as safe (GRAS) chemicals and other products like some essential oils, plant extracts, and other natural compounds, synthetic inorganic or organic salts such as carbonates, bicarbonates, sorbates, benzoates, acetates, paraben salts, silicates, etc. are exempt from residue tolerances on all agricultural commodities by the United S Food and Drug Administration. The advantages of these salts are their great availability, ease of handling and use, and low cost and they used to manage postharvest diseases of fruits and vegetables. A wide range of metals and their oxide-based single- and multi-walled carbon nanotubes and nanocomposites have been used efficiently as antimicrobial agent in fresh products. Gold, silver, zinc, cerium, titanium dioxide (TiO₂), silica, silica–silver, alumina–silicate, and chitosan are nanoparticles used to control plant pathogens. The fungicidal activity of TiO₂ nanoparticles has been proven against fungal pathogens *Venturiainaequalis* and *Fusarium solani*. The inhibition of pathogens *Botrytis cinerea* and *P. expansum* by using nanoparticles of zinc oxide is due to the induction of reactive oxygen species. Similarly, growth of *B. cinerea* and *C. gloeosporioides* is suppressed in solution of silica–silver.

The development and use of resistant genotypes are one of the major methods of preventing postharvest diseases in fruits and vegetables. But, little attention has been paid to develop resistant

varieties against postharvest pathogens in fruits and vegetables. In general, those varieties are preferred by people, which have thin peel, have low tannin content and high sugar content, and unfortunately, all these factors favour susceptibility to postharvest pathogens. Plant breeder needs to recognize the resistance to postharvest diseases, which is different from the field resistance. Hence, it is need of hour to develop such a breeding programme which meet the requirement of disease management. Genome editing technology has better efficiency to change the genome architecture at precise locations, with appropriate accuracy. It must be exploited alongside the conventional ones that have been limiting success. Due to their effectiveness and specificity of cleavage recognition sites, either by RNA-directed SDNs or protein-directed SDNs, site-directed nucleases (SDNs) utilized in plant editing technologies are frequently used to modify genomes. It includes meganucleases, ZFNs, TALENs, and CRISPR/Cas used to achieve random, predicted, or precise insertion. VvWRKY52 gene functioning as carotenoid biosynthesis pathway is used through CRISPR/Cas9 system to develop resistance against *Botrytis cinerea* in grape. In vegetable, *Capsicum annuum* L., *C. annuum* ethylene-responsive factor 28 (CaERF28) gene functioning as Annuum anthracnose pathogen (*Colletotrichum truncatum*) resistance is used through CRISPR/Cas9 system to develop resistance for anthracnose disease.

The recent trend is shifting toward safer and more eco-friendly alternatives to control postharvest decays in horticultural produce. Of various biological approaches, the use of antagonistic microorganisms is becoming popular throughout the world. Basic approaches to using microorganisms in postharvest disease control include managing beneficial microflora present on the fruit surfaces or artificially inoculating antagonists against postharvest pathogens. Biological control using artificially introduced antagonists is one of the most effective methods to prevent disease through competition for nutrients, antibiosis, the production of secondary metabolites such as volatile organic compounds, lytic enzymes, and the activation of the plant defense system, they function as antagonists of fungal diseases. *Debaryomyces hansenii*, *Galactomyces geotrichum*, *Pichia kudriavzevii*, *Rhodotorula glutinis*, and *Schwanniomyces vanrijiae* isolated from apple fruit, *Proteobacteria*, *Actinobacteria*, and *Bacilli* isolated from banana, guava, mango, papaya fruits, *Metschnikowia*, *Hanseniaspora*, *Acinetobacter*, *Gluconobacter* isolated from grape berries and *Lactobacillus plantarum* CM-3 in strawberries are used as microbial antagonists through the synthesis of phytohormones and volatile compounds inhibiting postharvest fungal pathogens. Yeast *Candida tropicalis* (IFRPD 6010) reduced spore germination and mycelial growth of *Lasiodiplodia theobromae* by competing for nutrients with the conidia of the pathogen. *Pseudomonas synxantha* isolated from kiwi fruit significantly inhibits growth of *Monilinia fructigena* and *M. fructicola* causing diseases in stone fruits. Similarly, *Lactococcus lactis* and *Weissella cibaria* inhibit *Erwinia mallotivora* and reduce papaya fruit disease. Antibiotics (iturin, pyrrolnitrin, and syringomycin) produced by bacteria are a heterogeneous group of low molecular weight organic compounds which inhibit the growth of *A. flavus*, *A. solani*, *F. oxysporum*, *C. gloeosporioides* by disrupting the structure of the cell wall or the function of the membrane of microorganisms, disrupting the synthesis of proteins and the function of respiratory enzymes. *Bacillus subtilis* produces antibiotics and volatile organic compounds to suppress postharvest pathogens such as *B. cinerea*, *R. stolonifer*, and *Colletotrichum* spp. in strawberries and *Penicillium italicum* and *P. digitatum* in orange. The extracts of *B. subtilis*, *Pseudomonas brenneri*, and *P. koreensis* significantly inhibit germination and hyphae growth of *B. cinerea* and *A. alternata* in blueberries by producing metabolites like arthrofactins.

Bacteria indirectly activate local and systemic responses in plants. Antagonistic microbes induce genes related to L-phenylalanine metabolism, amino acid biosynthesis, plant hormone signal transduction, and programmed cell death regulation. *B. siamensis* decreases the expression of catalase and increases the expression of superoxide dismutase (SOD). Increasing SOD activity can enhance defense in the host against pathogens. *C. oleophila* and *Meyerozyma guilliermondii* are found effective to inhibit *P. expansum* and *B. cinerea* kiwi and *P. italicum* and *P. digitatum* in orange after

harvest. *Pichia membranefaciens* yeast has a significant effect against *Rhizopus* rot of peaches due to induction of defense enzymes, such as CAT, POD, PAL, and PPO. *P. membranefaciens* induced mitogen-activated protein kinase cascade signaling pathway and ethylene, jasmonate, and salicylic acid signal transduction pathways to regulate transcription factors.

Application of essential oils (clove, cinnamon, and thyme oils) and plant extracts is an eco-friendly and sustainable method for postharvest disease management. Essential oils disrupt cell membrane integrity of fungi or defunct mitochondria, vacuole, and inhibit efflux pumps. The synergistic effects of the constituents of essential oils reduce the possibility of pathogen survival or resistance. Pomegranate peel phenolic extracts stimulate expression of PAL, chitinase, chalcone synthase, and mitogen-activated protein kinase, which contribute to the activation of plant defenses for response to reactive oxygen species and resulting inhibiting the germination of conidia of *P. italicum* and *P. digitatum*. Applying of clove and thyme oils inhibit conidia germination and mycelia growth of mango anthracnose pathogen. Thymol fumigation reduces anthracnose disease in avocados. Thymol inhibits pomegranate fruit rot by disrupting the function of cell-wall-degrading enzyme (cellulase and pectinase) of fungi.

In general, waxes and coatings are primarily applied to the fresh fruits and vegetables to increase their shelf life by regulating the exchange of water and gases (oxygen and carbon dioxide) and allows for less weight loss during storage. In addition, coatings provide shine and gloss to enhance external appearance of the produce. Besides natural coatings such as chitosan and *Aloe* spp. gels, most coatings are synthetic formulations containing blends of hydrocolloids (proteins or polysaccharides) and lipids (waxes, acylglycerols, or fatty acids) as constituents of the composite coating matrix. These main ingredients are typically formulated with plasticizers such as sucrose, glycerol, sorbitol, propylene glycol and emulsifiers (fatty acids, polysorbates, monostearates, lecithin) to enhance the coating integrity and emulsion stability, respectively. Resins such as shellac are also often added to provide gloss. Furthermore, additional ingredients such as some GRAS salts acting as antimicrobial agents can be incorporated to reduce decay during storage.

The effective management of postharvest diseases of fruits and vegetables is a very difficult task. The main emphasis should be given on proper diagnosis and detection of diseases and pathogens, exploring the possibility and potentiality of use of microbial antagonists against major pathogens, identification of compounds, which applied into the trunk or sprayed on foliage 15 -30 days before harvest, are translocated to the fruit, making it resistant to the postharvest pathogen, genome editing of target gene, identification of genes that promote epiphytic antagonists and producing resistant fruits with good quality characters. It is essential to ensure that close coordination, constant surveillance, and efficient technical support for rapid detection and precise identification of microbial pathogens, and feedback on the effectiveness of corrective measures taken to minimise the incidence and subsequent spread of the diseases, are available. Avoidance of wounds during postharvest handling of the produce and proper packaging is the basic precaution, to be strictly enforced in all the cases. The strategies that can directly act on the postharvest pathogens may be integrated broadly with different control methods like physical, chemical, biocontrol, and resistance in different combinations. The possibility of integrating the different effective strategies to achieve higher level of control of postharvest diseases. The usefulness of integrating different strategies starting from pre-harvest stage to provide better control of the diseases and get safe, disease- and residue-free fruits and vegetables is the need of hour.

Dr. Dinesh Singh
Head & Principal Scientist
Division of Crop Protection
ICAR- Indian Institute of Sugarcane
Research, Lucknow – 226002
Email: dinesh_ari@rediffmail.com