Agri Articles
(e-Magazine for Agricultural Articles)
Volume: 06, Issue: 01 (JAN-FEB, 2026)
Available online at http://www.agriarticles.com
©Agri Articles, ISSN: 2582-9882

Control of Postharvest Fungal Diseases in Fruits using External Application of RNAi

*Preeti Vashisht[1], Pooja[1] and Narender Kumar[2]
[1]CCSHAU, College of Agriculture, Bawal, Rewari, Haryana, India
[2]CCSHAU, KVK, Bawal, Haryana, India
*Corresponding Author's email: dhimanpreeti45@gmail.com

The availability of fruits and vegetables is decreased by postharvest losses of fresh vegetables, which are estimated to be between 20% and 40% worldwide. The forecast that there may not be enough food produced today to feed everyone on the planet by 2050 worries a number of international organizations. Therefore, there is an urgent need for postharvest loss reduction techniques to ensure food and nutritional security. The scenario is the same in Brazil. Lack of technologies, such as continuous cold chain and extensive handling of the fresh food, contributes to considerable postharvest losses. A person's diet should include a lot of fresh fruits and vegetables. However, these are perishable after harvest and microorganisms like fungi (*Alternaria*, *Aspergillus*, *Botrytis*, *Ceratocystis*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Geotrichum*, *Lasiodiplodia*, *Monilinia*, *Mucor*, *Penicillium*, *Pestalotiopsis*, *Phoma*, *Phomopsis*, *Phytophthora* and *Rhizopus*), bacteria (*Erwinia*, *Pseudomonas*, *Xanthomonas*, *Acetobacter*, and *Enterobacter*), and yeast (*Candida* and *Saccharomyces*) can cause them to spoil due to mechanical damage during and after harvest (Fig 1). In the agricultural industry, this is one of the primary reasons for worldwide economic losses.

Chemical fungicides are frequently used to prevent fresh produce from decomposing after harvest as a result of fungal growth. But these compounds' continuous and extensive usage is raising questions about their long-term impacts on human health and the environment, necessitating the development of new alternatives. The application of innovative fungicides utilizing RNA interference (RNAi) technology, which has higher specificity and efficiency, is one of the most recent environmentally and health-friendly tactics. A promising method for managing phytopathogens is the external application of double-stranded RNA (dsRNA), small interference RNA (siRNA), or hairpin RNA (hpRNA) to induce post-transcriptional gene silencing. The purpose of this study is to go over recent observations and research on the external application of RNAi-based fungicides to prevent postharvest spoiling.

RNAi Silencing Techniques to Control Postharvest Fruit Diseases

By introducing dsRNA molecules with sequences complementary to the target messenger RNA (mRNA) into the cell (Figure 2), RNAi contributes to a naturally occurring gene silencing process that is intrinsic to eukaryotes highly conserved, and crucial for the control of gene expression, genome stability, and heterochromatin formation. RNA-dependent RNA polymerase (RDR) forms this dsRNA from single stranded RNA (ssRNA) molecules, which may or may not have hairpin dsRNA stretches. Cloned portions of the target gene can also be used to create dsRNA in vitro. Either electroporation or microinjection can be used to introduce it into the cell. Once within the cell, the lengthy dsRNA is recognized and broken down into smaller, around 20–24 nucleotide siRNA by the highly specialized ribonuclease Dicer of the RNase III family.

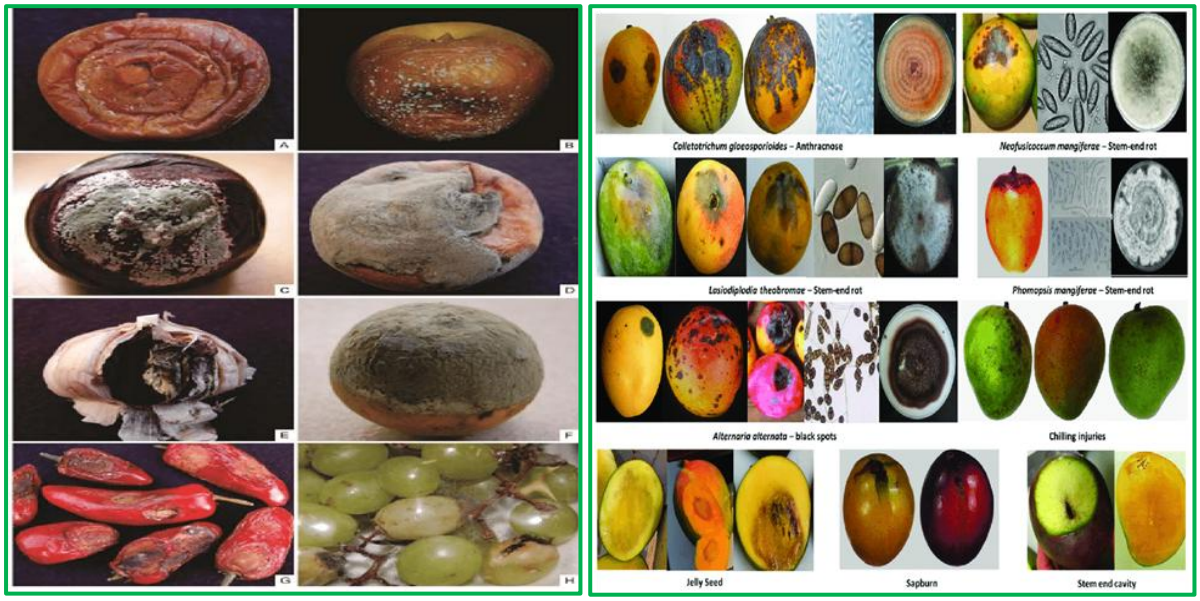

Fig 1. (Source: Research Gate)

As they come together with protein components to form an RNA-induced silencing complex (RISC), which includes Argonaut proteins (AGO) with three specific domains to engage with siRNA and identify the target mRNA molecule, they unwind into ssRNA. The RNA-induced transcription silencing complex (RITS) is formed when the activated RISC complex (i) methylates the histones attached to the genome by interacting directly with the gene of interest; (ii) binds to complementary transcripts by base pairing between the siRNA antisense strand and the target mRNA, causing its cleavage and eventual degradation, preventing its translation (iii) transforms transcription products into dsRNA. A transcript interference-based silencing technique known as post transcriptional gene silencing (PTGS) is based on the host-induced gene silencing (HIGS) and spray-induced gene silencing (SIGS) techniques, which are used to control agricultural pathogens. The RNA interference mechanism can affect the degradation of mRNA or directly on the repression of gene transcription (Figure 2). In order to control fungi like Fusarium graminearum in agricultural products, the HIGS technique is used for gene silencing with RNAi. In one instance, tobacco plants were modified to express dsRNAs complementary to the mRNA of the β-glucuronidase (GUS) gene of a transgenic strain of Fusarium verticillioides. In one instance, the plant was genetically modified to endogenously produce the dsRNA molecules specific to the pathogen's target genes.

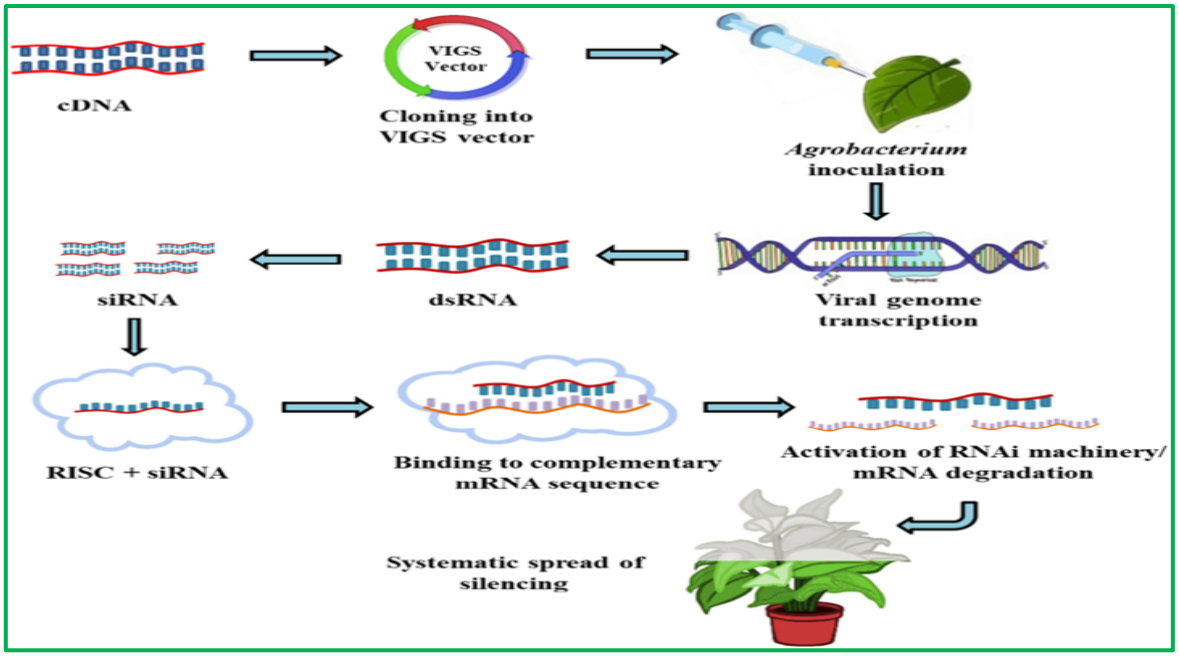

Fig. 2 (Source: link.springer.com)

The target gene was silenced by the siRNA molecules that were created when the dsRNA was integrated into the fungus during inoculation. The HIGS mechanism targets the genes involved in the host's response and interaction with diseases and pests by causing the host to express hairpin or short RNAs. This results in genetically modified organisms (GMOs), which end users often reject. The main issues with the modified RNAs' broader use include their difficulty in application, their instability following target gene silencing, and the absence of protocols for executing genetic alterations in several species. Without the endogenous expression of dsRNA and siRNA, SIGS technology has become a viable alternative to HIGS for controlling postharvest fungus. Since the RNA molecules are on the plant's surface, external application of dsRNA and sRNA to the plant has significant antifungal potential. (Fig 3)

They can either be directly absorbed by the fungal pathogens or absorbed by the plant cells and then transferred to the pathogens. The SIGS method is quick, effective, and ecologically friendly. It is based on the direct spraying of dsRNA onto the surface of plants or fruits and does not require transgenic modification. The interference process is started by recruiting RNAi machinery once it enters the pathogen's cells. Despite the fact that dsRNA transport differs from organism to organism, fungal cells can use one of two routes. After entering the plant cell, the dsRNA molecules use its silencing machinery to produce siRNA molecules, which subsequently move to the fungus. Alternatively, it may be directly taken by the fungus and attract the pathogen's own cellular apparatus.

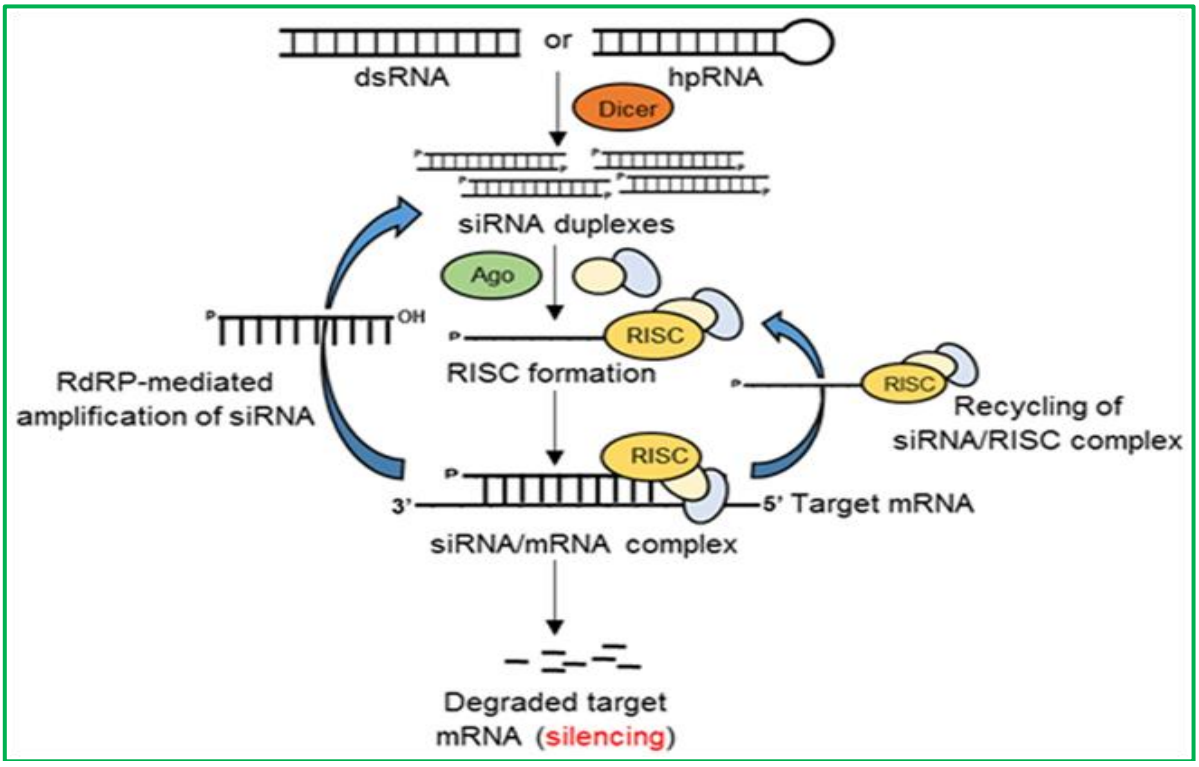

Fig 3 (Source: Frontiers)

Biofungicides Based on RNAi and Gene Silencing to Control Postharvest Fruit Pathogens

Because it does not require transgenic modification, employs specific sequences, and enables action on highly specific targets, the external application of RNA interference (RNAi) is a promising alternative to the fungicides commonly used to control the disease in the field and during postharvest handling. Since the fruits are not exposed to harsh environmental conditions like sunlight, which helps to reduce dsRNA degradation and provide long-lasting protection during postharvest, the use of dsRNA for the control of postharvest fruit diseases represents a new generation of sustainable and environmentally friendly products. The direct spray technique has been used to study the external administration of RNAi for the

management of postharvest illnesses in the postharvest stage of fruits (**Figure 4)**. Topical application of RNA interference (RNAi) has already been studied in relation to fruit postharvest fungus, particularly *Fusarium* spp. and *Botrytis cinerea.* Gray-mold disease is caused by the fungus B. cinerea, which affects over 200 species worldwide. It is regarded as one of the primary pathogens that cause postharvest losses in a variety of crops, including cucumber, strawberry, tomato, lettuce, and grape, resulting in large financial losses. Due to challenges in controlling infections brought on by members of this genus, *Fusarium* spp. generate large economic losses in the agricultural sector globally. Fruits like oranges, muskmelon, bananas, and kiwis are susceptible to postharvest infections caused by species of the Fusarium genus, including *F. oxysporum*, *F. solani*, *F. incarnatum*, and *F. musae.*

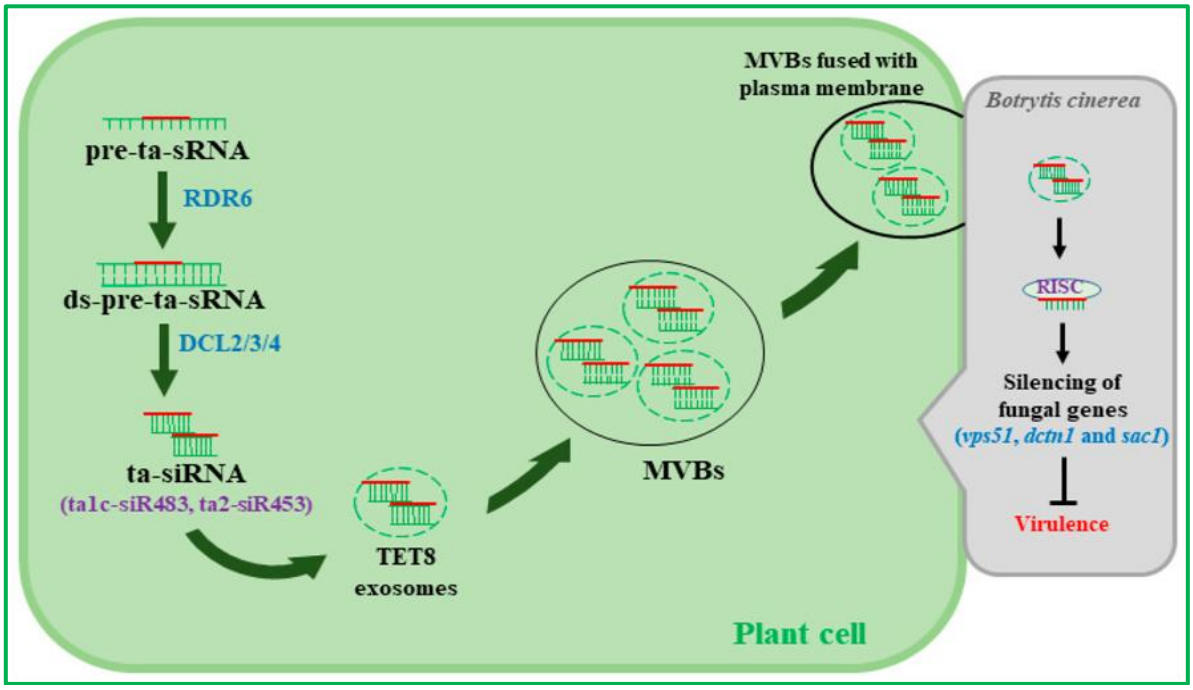

Fig 4. (Figure: MDPI)

The Bc-DCL genes were silenced by the genetic alterations of these RNAs, which also reduced the virulence and development of *B. cinerea*. Gray-mold disease was prevented by applying dsRNA and ssRNA molecules to the surface of tomato (*S. lycopersicum* "Roma"), strawberry (Fragaria × ananassa), and grape (*Vitis labrusca* "Concord") plants. The fungus B. cinerea's capacity to evade the host plant's immune system—which has been documented for *Arabidopsis* and tomatoes—can be linked to the mechanisms behind the application of exogenous RNA interference. In this study, the authors silenced the *AGO1* gene in tomato and *Arabidopsis*, needed for RNAi process. These mutant plants exhibited reduced susceptibility to the disease after being infected with the fungus. The virulence of a mutant strain of B. cinerea that had its DCL1 and DCL2 genes silenced, which are necessary for the synthesis of sRNAs that change a plant's immunological defense, was significantly reduced in tomato and *Arabidopsis*. The primary β-tubulin that is necessary for the growth of certain fungus is β2-tubulin. RNAi molecules derived from various regions of β2-tubulin had a direct impact on asexual reproduction, mycelium growth, and virulence. This suggests that a new fungicide could be investigated to control postharvest spoilage, as it is a safer and more effective alternative to chemical fungicides like carbendazim. It was previously unknown how siRNAs/dsRNAs move between organisms from different taxonomic kingdoms, such as from plants to fungi and/or vice versa, because the movement of siRNAs through plasmids to neighboring cells and through the vasculature system to distant parts of the plant is well established.

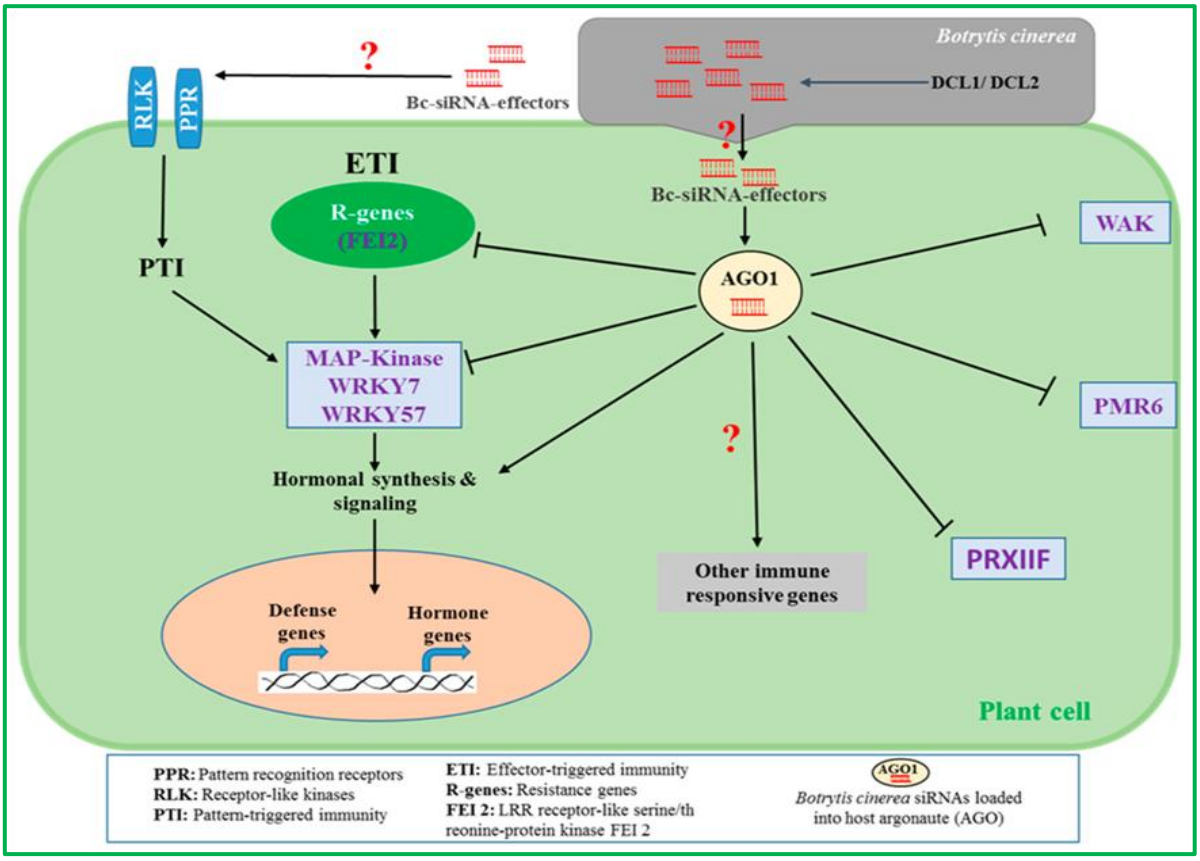

Fig 5. (Source: MDPI)

It was demonstrated that exosome-like extracellular vesicles are secreted by *Arabidopsis* to transport RNA molecules to B. cinerea, indicating a broader process in which exosomes play a role in transnational RNA transport. Accordingly, fungal RNA may also be delivered via exosomes, a multiprotein intracellular complex that may break down different kinds of RNA molecules. In order to make the approach unique to each disease and the hosts it impacts, it is crucial to investigate the genes of both hosts and pathogens in addition to creating new methods for applying RNA interference.

Target genes that effectively fight the particular fungus have been found in a number of research. New alternatives to traditional techniques for managing fungi in the field and throughout the postharvest phases have emerged as a result of the RNAi approach in agriculture. Because PTGS procedures are more specialized, they are not only more sustainable but also more effective. Strategies include employing nano encapsulation can increase the efficacy, stability, and resilience of dsRNA and siRNA against abiotic variables like ultraviolet (UV) radiation, oxygen, and temperature fluctuations in the postharvest products **(Figure 5)**.

By shielding dsRNA molecules from nuclease degradation and enhancing target cell transport, nanoencapsulation has been utilized to increase the effectiveness and durability of RNA interference (RNAi) without compromising its capacity to silence genes that target phytopathogens. Using nanomaterials that promote endosomal escape or conjugating nanoparticles with cell penetrating peptides can increase the effectiveness of dsRNA delivery in the cytoplasm of cells. Molecules having a variety of uses and functions, including metals, lipids, and cationic polymers, can be used to create nanoparticles for the transport of RNA interference.

Nanoparticles have been investigated in the context of RNAi-based crop protection to shield dsRNA molecules from environmental nucleases and UV radiation, which can break down unprotected dsRNA molecules. However, as far as we are aware, no research has been done on the use of microencapsulated RNA interference to prevent postharvest fruit illnesses. In other systems, RNAi nanoencapsulation has proven to be a viable method for disease management. Fruits and vegetables are given edible coatings to extend their shelf life. They

are made from biopolymers such proteins, lipids, and polysaccharides and are described as a thin layer that forms on the surface of food and can serve as a barrier to prevent water loss, gas diffusion, and changes in scent. These are primarily applied to the food surface by spraying or submersion.

Apart from providing physical protection, the coatings have the ability to transport a variety of bioactive substances and serve as a secure and efficient means of RNAi protection. The potential of polysaccharides like chitosan, hyaluronic acid, and its derivatives as RNAi carriers has been documented, despite the fact that we are unaware of any research on the use of RNAi linked to coatings in fruit or vegetable conservation. After 96 hours of incubation with HepG2 cells, chitosan carriers containing siRNA were able to 40% effectively mute SR-B1 and lower VEGF levels in an in vitro murine melanoma model.

Conclusions and Future Perspectives

As a safer substitute for synthetic fungicides, RNA interference (RNAi) has become a novel technique for controlling fruit deterioration after harvest. SIGS is a more acceptable option than genetic changes, which need approval from many regulatory bodies, because it does not entail genetic transformation like the HIGS procedure does. Because SIGS is simple to apply, it can effectively reduce fruit and vegetable postharvest rotting. To find genes crucial for the biotrophic and necrotrophic stages of fungal infections, comprehensive transcriptome investigations are required, as there is currently a dearth of knowledge regarding target genes for RNAi-based fungicides.

It is yet unknown how the fruit or fungal cells absorb the externally delivered dsRNA. The instability of dsRNA molecules in storage settings is another issue. Polymeric nanoparticles and edible coatings might work better to guarantee these compounds' increased stability and controlled, progressive release. Last but not least, future research must concentrate on the synthesis and stability of RNAi-based fungicides as well as their application for gradual release, as this technology has a lot of potential to minimize postharvest fruit loss.