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ASSOCIATION OF MICROBIAL DIVERSITY WITH POST HARVEST CROPS AND BIOPROSPECTING OF ENDOPHYTIC MICROORGANISMS FOR MANAGEMENT

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ABSTRACT

While thinking about the bigger scenario of global food security, loss of food materials during post harvest period is bigger challenge. About one third of food products get wasted before reaching the consumers stomach and this loss is big in tropical and less developed countries. Damage of harvested food crops and stored crop produce by microbial infestation shares a quite big space in this regard. Crops may be infected by pathogenic microflora in pre-harvest stage and then may flourish and damage the food materials in post harvest period especially in the storage. Most of the time these microbial association leads to the deterioration of food crops like fruits, vegetables and cereal grains

which causes great economic loss. Microbial pathogens like *Botrytis, Penicillium, Sclerotium, Colletotrichum, Dothiorella, Phytophthora, Pythium, Rhizopus* and *Xanthomonas* cause various rots, spots in many fruits and vegetables. *Aspergillus, Penicillium, Fusarium* species are largely involved in the production of mycotoxins mostly in cereal grains. Long term control of the post harvest pathogens with the application of synthetic chemicals is never the best of idea. Endophytic microorganisms (bacteria, fungi and actinomycetes) which live asymptomatically inside plant tissues possess the potential to inhibit a broad spectrum of these pathogens. Antagonistic activities of endophytes like *Muscodor, Trichoderma, Penicillium, Phoma, Phaeosphaeria, Bacillus, Pseudomonas, Streptomyces,* etc. have been well explored by many researchers throughout the world. The current review has highlighted the enormous potential of this important microbial group to control the post harvest pathogens including storage pathogens. The research gaps in this area has also been indicated while the need for carrying out advanced research and devising suitable management strategies using endophytes has been brought forward.

Keywords: *Post harvest, Endophytes, Antagonists, Mycotoxin, Rots, Aspergillus, Fusarium, Storage Fungi, Cereal, Fruits and Vegetables.*

1. Introduction

According to the FAO, food production will need to grow by 70% to feed world population which will reach 9.1 billion by 2050 [38]. When talking about the global food security in this scenario of growing global food demand, food loss due to various reasons possesses a great challenge to the food scientists, policy makers and food handlers. Many recent information endorsed by numerous scientific investigations have foregrounded the evidence of loss of food materials which might start at field condition (due to biotic and abiotic stresses), post harvest and until the moment of consumption. Roughly about 1.3 billion tons (one-third of global production) of food produced for human consumption is lost or wasted from food chain globally which worth about US $1 trillion [31,59]. Among different agricultural commodities, the studies estimated that on a weight basis, cereal crops, roots crops, and fruit and vegetables account for about 19%, 20%, and 44% losses respectively [53]. Half of the horticultural crops alone are lost due to post-harvest activities such as harvesting, handling, storing, processing, packaging, transporting and marketing [45]. The amount of loss is higher at consumer end in medium and high income countries while it is higher at the early stages of the post-harvest system in developing or low income countries [83]. Postharvest loss may be defined as the measurable qualitative and quantitative loss of food material along the supply chain which might start at the time of harvest till its consumption or other end uses [50]. The losses can broadly be

categorized as weight loss due to spoilage, quality loss, nutritional loss, seed viability loss and commercial loss [13]. Many post harvest diseases caused by microbial pathogens like bacteria, fungi etc. also contributes a substantial part in this type of loss though precise estimation of the exact quality and quantity has not been worked out globally. Still a significant portion of harvested produce, especially in less developed countries, has been unable to reach the consumer due to the spoilage by post-harvest diseases. The science of this second phase of plant protection which basically deals with the protection of harvested produce during harvesting, packing, transporting, processing, storing and distribution is known as "Postharvest pathology", earlier termed "market pathology". Microbial pathogens could make their way in to the crops before harvesting and subsequently proliferate during transit and mostly in storage period to cause post harvest diseases [71]. Crop loss due to postharvest diseases can go up to 10–30% [3] where in developing countries and tropical regions loss of perishable commodities can be as high as 50% [110].

Food security is the biggest challenge in current scenario and stress imposed by disease causing pathogens has been immersed as an ever growing problem. Hence eco-friendly approaches by utilizing friendly microbes need to be thought of to overcome the hurdle. Keeping this in the back of mind the current review discusses about the post harvest problems in various food crops caused by different pathogenic fungi. Simultaneously, the potential and bio-prospecting of endophytic microorganisms to mitigate the problem has also been put forward.

1.1. Implications of Post Harvest Diseases of food crops: Why and How?

The occurrence of post harvest diseases is preliminarily depends on the type of commodity and its susceptibility to pathogen infection. Microbiological spoilage is a bigger threat to perishable food crops like fruit and vegetables which contain higher moisture levels (about 50% or more). In contrast, durable or less perishable crops like roots, tubers and food grain products, cereals etc. which are generally stored at lower moisture content (below 12%) have comparatively less chance of microbial invasion. However, microbial pathogens may make their way into the grains when the crops reach physiological maturity and grain moisture contents reaches 200-300 g/kg. In addition to that standing crops may get damped by unseasonal rains which subsequently lead to the lodging of plants which creates further opportunity for soil pathogens to enter into the grains. Finally under poor storage conditions and mishandling of harvested produce, the

hidden microbes may proliferate and numerous species of storage fungi and bacteria may contaminate a wide variety of cereal grains which might cause quantitative and qualitative losses [48,71]. Post harvest environments like optimum temperature, adequate relative humidity and conducive atmospheric composition always encourage the growth of post harvest disease causing fungi and bacteria. Susceptibility of crop genotypes and maturity or ripening stage is also vital for disease infection [23].

1.2. Post Harvest Microbial Pathogens

Microorganisms present on growing plants without causing disease symptoms in, but only cause symptoms in harvested plant products may be considered as post harvest pathogens. However a true post harvest pathogens may be considered as which do not infect plants in the field but present on harvested products where they can cause disease [112]. Research on the postharvest microbiota of crops, their dynamics and impact on storage stability has not been carried out extensively. Diseases occurring in the crops after harvest are generally caused by fungi and bacteria, though in very few cases some virus may be responsible which cause progressive reduction in yield and quality of the produce. However, fungi are known to be prolific disease causing agent in post harvest process where more than 100 species are responsible for the majority of postharvest diseases [110]. Most of them belong to the phylum Ascomycota and the associated fungi Anamorphici (Fungi Imperfecti). Important genera of anamorphic postharvest fungal pathogens include *Penicillium, Aspergillus, Geotrichum, Botrytis, Fusarium, Alterneria, Colletotrichum, Dothiorella, Lasiodiplodia* and *Phomopsis*. Among others the genera *Phytophthora* and *Pythium* (Oomycota), *Rhizopus* and *Mucor* (Zygomycota), *Sclerotium rolfsii* and *Rhizoctonia solani* (Basidiomycota) can cause significant post harvest loss. Important bacterial species for the same cause include *Erwinia, Pseudomonas, Bacillus, Lactobacillus* and *Xanthomonas* [23].

It has been found that the disease development is strongly influenced by the endophytic microbial community though the pathogens trigger pathogenicity. Some researchers have also pointed out towards the shift in microbial community on crops during disease development e.g changes in the bacterial community composition of potato tubers in response to infection with the soft rot pathogen *Pectobacterium atrosepticum*. Similarly, shifts in the fungal and oomycete community composition associated with storage soft rot development in different sugar beet hybrids from different environments stored at different temperatures has also been reported [16,57].

1.3. Deterioration of harvested produce by Storage Fungi

Estimated losses of seeds, especially staple cereal grains in store from all causes may amount to 10% worldwide but can reach 50% in tropical regions [60]. Deterioration of stored grains by fungal pathogens have been reported almost 100 years ago but since the last 5-6 decades this problem has been revived once again due to the growing food demand [21]. Fungal species mostly belonging to genera *Aspergillus* and *Penicillium* etc. relatively active more in storage conditions are known as Storage fungi or storage moulds. These groups of pathogens generally make way to harvested grains and stored crop produce through contamination of spores that might be present on spilled grains present in harvest, handling and storage equipment or structures. As suggested by Neergaard (1977), storage fungi can be recognised into five groups based on conidial morphology and spore-producing structures: (1) hyaline fungi producing thin-walled conidia such as *Botrytis*, *Cercospora*, and *Fusarium*; (2) strongly pigmented fungi with thick walled conidia such as *Drechslera* and *Alternaria*; (3) fungi producing acervuli or pycnidia which protect the conidia formed inside as in *Colletotrichum* and *Botryodiplodia*; (4) fungi producing deep-seated resting mycelium and heavily pigmented spores as in smut fungi; and (5) resting mycelia of internally seed borne fungi [71]. Various types of adverse effects can be induced by storage fungi on durables, such as reduction in germination, discoloration, musty or sour odors, caking, nutritional alterations and reduction in processing quality all of which gradually make a “Hot Spot” in storage bulk [115].

Microbial deterioration of grains can be broadly determined by four biological factors: **a.** intrinsic factors depend on the characteristic and nature of the growth substrate, **b.** extrinsic or external factors, **c.** processing factors (all agricultural operations, where the composition of the microflora is primarily modified) and **d.** implicit factors (virulence of dominant microbial flora that initially develop in response to all other factors) [60]. Among all favourable environmental conditions, higher content of moisture always encourages the growth of storage fungi. Unlike field fungi which require moisture content in equilibrium with relative humidity of 95 to 100% for growth, storage fungi can’t colonize actively metabolizing plant tissues but can grow without free water. The range of water activity allowing fungal growth is between 1.00 (pure water) and about 0.6. Moisture content below 13.5 percent in starchy cereal seeds such as wheat, barley, rice, corn and sorghum and below 12.5 percent in soybean prevents invasion by storage fungi regardless of how long the grains are stored [108]. However, moisture content as low as 6.5% in

Sunflower and 7.3% in Celery might be sufficient for initiation of fungal growth [21,93]. As far as temperature is concerned the majority of spoilage causing fungi in stored grain ecosystems thrives over the range 4 to 15^0C to 30 to 55^0C, with optima in the range 25 to 35^0C. When stored produce receives moisture in small pockets, it can allow the initiation of fungal activity that produces metabolic heat resulting in a succession of dominant fungi which gradually ends with spontaneous heating and dominance by thermotolerant or thermophilic fungi and actinomycetes [56]. In general, survival of the storage fungi is progressively reduced with increase in storage period irrespective of storage temperature. The storage fungi are generally considered to be obligate aerobes while many of them are also micro-aerophillic, being able to survive in niches having Oxygen concentration as low as 4% and more than 80% of CO_2. Successful proliferation of storage fungi is also enhanced when the grains are broken due to mishandling and injured due to insect attack. Further, when the amount of insects and mites in grain carrying fungal spores on their bodies increases, the introduction of storage fungi into the new and unaffected grain mass accelerates. Aggressive activity of insects and mites in a grain mass gradually leads to an increase in both temperature and moisture content of the grain resulting in 'hot spots' where mold growth is encouraged [55,60,71,108].

1.4. Microbial deterioration of harvested and stored Cereal produce

Billions of people around the world depend on rice, wheat and maize, and to a lesser extent, sorghum and millets as their staples for daily survival. More than 50% of worldwide daily caloric intake is derived directly from cereal grain consumption [7]. Though cereal grains are considered as durable food products but it can also be damaged by microbial pathogens under certain conditions. As discussed in the previous section, the storage environment is very vital for such purpose. Significant damage may be carried out by storage fungi especially the production of mycotoxins in the edible grains holds a great threat to food security. Detail discussion on this issue shall be carried out in the forthcoming section. Other than that many cereal seeds and grains after harvest could be damaged by microbial contamination under storage, however, milling of grains after harvest may remove significant microbial load. While storage pathologists and microbiologists have given adequate attention to post harvest study of the seeds of barley, maize, wheat and other cereal crops, comparatively little work has been done for rice, a staple food for more than half of the world's population. Greater microbial diversity was observed in fresh paddy as compared to milled rice but dominant fungi and bacteria were specifically

present or enriched during storage. Bacteria belonging to the genera *Bacillus, Pectobacterium, Pantoea, Microbacterium, Sphingomonas, Methylobacterium, Enterococcus, Pseudomonas, Rhodococcus Enterobacter, Xanthomonas, Cellulomonas, Clavibacter, Burkholderia, Lactobacillus, Lactococcus, Leuconostoc, Weissella, Serratia, Pseudomonas, Erwinia, Haererehalobacter, Clostridium, Curtobacterium, Chryseobacterium, Flavobacterium, Pedobacter, Staphylococcus, Exiguobacterium* and *Paenibacillus* have been found to be associated with fresh un-milled rice, stored milled rice, fresh milled rice and packaged rice [26,41,80,81]. Rice after harvest is also get infected by numerous fungal species like *Aspergillus, Fusarium, Alternaria, Cladosporium, Pseudozyma, Cryptococcus, Candida, Cyberlindnera, Phaeosphaeriopsis, Bullera, Pichia, Debaryomyces, Acremonium, Arxula, Rhizopus, Xeromyces, Trichosporon*, etc. [105109]. Misra *et. al*., (1995) has isolated number of fungal species from 50 stored rice samples collected from warehouses/ rice mills in Laguna area of phillipines. These fungi included *Alternaria padwickii*, *Aspergillus amstelodami*, *A. candidus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. repens*, *A. restrictus*, *A. terreus*, *Bipolaris oryzae*, *Chaetomium cochlidodes*, *C. cuneatum*, *C. junicola*, *C. globosum*, *Cladosporium* sp., *Curvularia geniculata*, *C. lunata*, *Fusarium moniliforme*, *F. semitectum*, *F. solani*, *Fusarium* sp., *Microdochium oryzae*, *Monilia sitophila*, *Mueor racemosus, Nakataea sigmoideum*, *Nigrospora oryzae*, *Penicillium citrinum*, *Pestalotia* sp., *Phoma* sp., *Phyllosticta glumarum*, *Rhizoctonia* sp., *Rhizopus* sp., *Sarocladium oryzae*, *Tilletia barclayana*, *Verticillium alboatrum*, *Verticillium* sp., etc. Infestation by *A. flavus*, *P. citrinum* and *Rhizopus* gradually increased with storage period.

Wheat and maize crop faces mycotoxin contamination as the main post harvest problem which is discussed in the subsequent section. Many of above fungal pathogens such as *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Rhizopus* spp, *Trichoderma* spp have been reported to infect wheat in different parts of the world during post harvest period. Wheat is the most important staple food of about two billion people (36% of the world population), the third most produced cereals after maize and rice which provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally [15,52]. Spatio-temporal analysis of some stored wheat in Tunisia revealed its association with various atypical fungal species such as *Alternaria* (28%), *Fusarium* (19%), *Penicillium* (19%), *Aspergillus* (14%), *Mucor* (8%) and *Rhizopus* (7%). Various other genera of fungi imperfecti, including *Ulocladium*, *Geotrichum*, *Chaetomium*, *Trichothecium, Paecilomyces, Aureobasidium* and *Chrysonilia* (anamorphic

Neurospora), and the Mucorales genera *Lichtheiia* and *Syncephalastrum* accounted for about 6% of the total microflora [11].

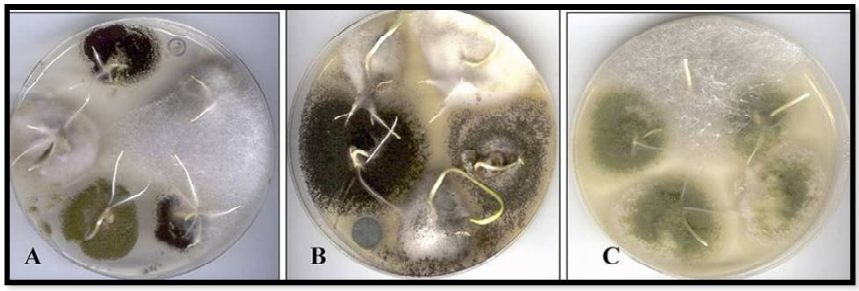

Fig-1: Emergence of storage fungi from stored wheat samples: (Courtsey: Belkacem-Hanfi *et. al.*, 2013).

In terms of production, maize is the leading crop in the world, with 825 MMT produced in 2010 where USA contributed 40% (Awika, 2011). *Aspergillus* sp., *Fusarium* sp., *Penicillium, Rhizopus* and *Bsyochlamys spp.* are the most predominant species attacking maize seed and resulting in reduction in seed germination and in most cases resulting in the production of mycotoxins [111]. Maize infected with ear rot complex has been associated with fungal flora like *Fusarium moniliforme, F. moniliforme* var. *subglutinans, Penicillium* species, *Stenocarpella maydis, S. macrospora* and *Acremonium* spp. [42].

Overall world production of sorghum and millet is much lower than the 'big three'. However, sorghum and millets are largely tolerant to drought and other abiotic stress and very important from nutrition point of view in parts of Africa and India, owing largely to their drought tolerance and other agronomic traits. Though produced in relatively in lesser quantity (60million MT of sorghum and 27million MT of millet in 2010) than rice, maize and wheat, about 50% of sorghum and 80% of millet production is used for human consumption [7]. In addition to routine mycotoxigenic fungi, sorghum has been associated with other post harvest fungal species which include *Cladosporium vignae* Gardner, *Macrophomina phaseolina* (Tassi) Goid and *Helminthosporium turcicum, Alternaria, Phoma, Curvularia, Penicillium, Drechslera, Mucor and Rhizopus* etc. [44,79,102]. Some farmer-saved sorghum seeds in Nigeria have been found to be contaminated with *Helminthosporium* sp, *Aspergillus* sp, *Fusarium* sp, *Rhizoctonia* sp, *Penicillium* sp, *Sclerotium* sp, and *Curvularia* sp. [1]. Fungal contamination has been found to be little higher in sorghum than millets. Eighteen seed borne fungal genera with 34 fungal species were identified from the seeds of South Korean sorghum samples and 13 genera

with 22 species were identified from the seeds of foxtail millet. Five dominant species such as *Alternaria alternata*, *Aspergillus flavus*, *Curvularia lunata*, *Fusarium moniliforme* and *Phoma* sp. were recorded as seed-borne mycoflora in sorghum and 4 dominant species (*Alternaria alternata*, *Aspergillus flavus*, *Curvularia lunata*, *Fusarium moniliforme*) were observed in foxtail millet [118]. Similarly 34 and 27 number of fungal species have been found in seed samples of sorghum and pearl millet, respectively in Burkina Faso. Fungal pathogens *Phoma* sp. and *Fusarium moniliforme* infected 95 to 100% of the seed samples of both sorghum and pearl millet [30]. Domination of microflora similar to that of other cereals has also been observed in another important cereal Barley which is grown in more than 70 million hectares in the world with a global production of more than 160 million tons [5]. Some older investigations reported the presence of numerous bacterial species on barley contaminated by remnants of rodents. Gram-positive bacteria like *Aureobacterium flavescens*, *Bacillus* spp., *Brevibacterium linens*, *Corynebacterium* spp., *Clavibacterium iranicum*, *Microbacterium imperiale* and *Oerskovia xanthineolytica* whilst gram-negative bacteria like *Erwinia herbicola*, *Pseudomonas fluorescens* and *Chromobacterium* sp. were abundant on such samples. In addition to this prominent mycotoxigenic species of *Aspergillus* and *Penicillium* have been the major contaminants [33].

1.5. Storage Pathogens and their Toxigenic Consequences

The previous section recapitulated information on the association of numerous bacterial and fungal species with post harvest cereal crops. Stored grain ecosystems are composed of dormant autotrophs, seeds, which serve both as an energy source and as habitat for many heterotrophic species of fungi, bacteria, insects and mites. Under storage conditions the microorganisms perform saprophytic activities the result of which an array of secondary metabolites are produced in the stored cereal grains. Most of these filamentous storage fungi are active producers of low-molecular-mass toxic secondary metabolite compounds known as mycotoxins [6,14]. The pre-harvested fungal flora may lead to the production of mycotoxins when the infected crop products undergo storage for a considerable period. A schematic diagram (Fig-2) developed by Fleurat-Lessard (2017) represents the factors for mycotoxins production in stored grains.

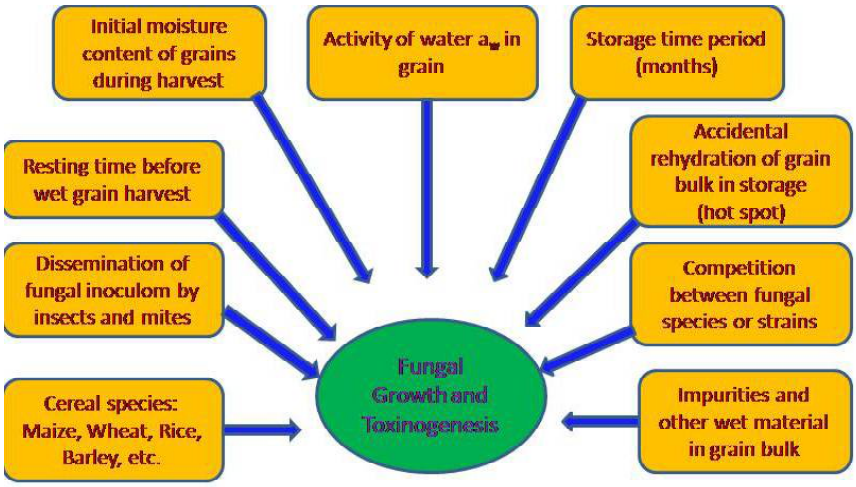

Figure-2: Key-factors involved in germination and growth of mycotoxigenic fungi developing in stored cereal grain during long-term storage: (Courtsey: Fleurat-Lessard, 2017).

Till date 300 different kinds of mycotoxins have been identified and reported which contaminate 25% of the world food crops leading to the loss of food and food products which cost around 1 billion tons per year [64]. Mycotoxins prevention has become important from the point of view of food security. Once developed in food products, mycotoxins become stable in the environment and very resistant to thermal changes including cooking, boiling, baking, frying, roasting, and pasteurization [97]. These low molecular weight chemical compounds are unpredictably harmful to human and animals even in very low concentrations and enter the human food chain via animal food products. Mycotoxins like aflatoxins, fumonisins, ochratoxins, trichothecenes, zearalenone etc. have been found to be carcinogenic by International Agency for Research on Cancer (IARC) [51,54]. Further these may have teratogenic, tremorogenic, haemorrhagic and dermatitis effect on vertebrates and consumption of contaminated plant foods or animal feed with mycotoxins, can lead to a number of metabolic problems such as liver function deterioration, protein synthesis interference or other disorders such as skin sensitivity, necrosis, or extreme immunodeficiency [107].

Contamination of Mycotoxin and mycotoxigenic fungi may take place to all crops such as cereals (maize, wheat, rice, barley, oats, and sorghum), peanuts, ground nuts, pistachio nuts, almonds, walnuts and cottonseeds. Maize has been the highest impacted crop, even some samples of maize silage have been found to be contaminated with 27 different

mycotoxins and other fungal secondary metabolites [22,103]. *Fusarium* sp. invade more than 50% of maize grain before harvest and produce mycotoxins. Though rice is the least affected cereal crop, many reports over the world and from India explored the association of mycotoxins and large number of mycotoxigenic fungi like *A. flavus, A. parasiticus, A. niger* and *A. ochraceus* in rice [72,91]. Post harvest stored wheat samples investigated by Birck *et. al*., 2006 have been detected with the presence of *Aspergillus* spp. in100%, *Fusarium* spp. in 80% and *Penicillium* spp. in 60% of the samples. Studies have shown that growth, mycotoxins production, competitiveness and niche occupation by mycotoxigenic species are influenced by the presence of other contaminant moulds and environmental factors. Contamination with mycotoxigenic fungi and mycotoxins leads to the deterioration of post harvest seed and grain quality kept under storage. The adverse effects include reduced germination, elongation of the hypocotyls or roots of developing seedlings, or both, and by interference with chlorophyll synthesis in certain plants, reduced seedling vigour, glume or grain discoloration, loss in viability and quality, heating and losses in nutritional value, production of off-odours, deterioration in baking and milling quality, etc. [27,43,73].

Most of the leading producers of mycotoxic fungi belong to five genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium* and *Fusarium*. There are also other genera, (viz., *Chaetomium*, *Claviceps*, *Diplodia*, *Myrothecium*, *Phoma*, *Phomopsis*, *Pithomyces* and *Stachybotrys*) that contain mycotoxin-producing fungi [77]. The main mycotoxins classes that occur in cereal are the aflatoxins (AFB1, AFB2 and AFG1, G2), the tricotecens, deoxinivalenol (DON) and (T-2 toxin), the fumonisins (FB1, FB2 and FB3), the zearalenone (ZON), ochratoxin A (OTA) and the ergot alkaloids [19,62]. Aflatoxins are produced mainly by *A. flavus*, *A. parasiticus* that are present normally in soil, agricultural products and various organic materials. Individual kernels of corn may contain as high as 400,000 ìg/kg of aflatoxin [120]. Ochratoxins are produced by *Aspergillus ochraceus*, *Penicillium verrucosum* and other *Penicillium* species in a wide variety of agricultural commodities such as corn, wheat, barley, flour, coffee, rice, oats, rye, beans, peas, and mixed feeds, and are notably present in wine, grape juice and dried vine fruits. Production of *Fusarium* toxins and *Fusarium* infection itself may start from the field. *Fusarium* species infect important crops such as soft and durum wheat, barley, oats, rice, maize, potato, asparagus, mango, grasses, and other food and feed grains [37]. Wheat, triticale, and maize grains are especially vulnerable to *Fusarium* infection and are also frequently more highly contaminated with their

secondary metabolites. A study by Kibe (2015) revealed the presence of *Fusarium poae, Fusarium verticillioides, Fusarium boothii* as the primary mycotoxin producing fungi contaminating some maize samples. *Fusarium verticillioides* was found to be predominant (33%), followed by *Fusarium boothii* (17%) and *Fusarium poae* (12%). Apart from that other common toxin producing species include *F. culmorum, F. graminearum, F. sporotrichioides, F. crookwellense, F. acuminatum, F. equiseti, F. proliferatum, F. armeniacum, F. pseudograminearum*, etc. Most harmful *Fusarium* toxins include Fumonisins (B1, B2), Deoxynivalenol, Zearalenone, T-2 toxin, Diacetoxyscirpenol, Moniliformin, Trichothecenes etc. [6,32,77,92,120]. Growth rate of *F. culmorum* has been observed to be significantly faster when interacting with *Microdochium nivale* and *Penicillium verrucosum* than when growing alone on grain [60].

Post harvest fungi causing mycotoxicoss is a huge problem in post harvest cereals. Effective control methods need to be devised and regulatory mechanism need to be followed for its prevention and contamination.

1.6. Post Harvest Microflora of Fruits and Vegetables

Worldwide consumption of fruits and vegetables has dramatically increased since the last few decades when alone United States witnessed an increase of 30% [10]. Fruits and vegetables contain higher amount of essential nutrients like vitamins, carotenoids, fibers, plant proteins etc. Common fruits produced worldwide include watermelons, bananas, apples, grapes, oranges, mangos, pears, plantains, peaches, pineapples, nectarines, lemons, papaya, plums, strawberries, avocados, kiwi fruit, apricots, cherries, raspberries, etc. Worldwide produced vegetables are tomatoes, onions, cucumbers, cabbages, brinjal, carrots, turnips, chillies, pepper, spinach, garlic, pumpkin, squash, guards, cauliflower, broccoli, beans, peas, okra, asparagus, etc. (www.statista.com). India has been the lead producer where 86.602 million metric tonnes of fruits and 169.478 million metric tonnes of vegetables were produced during 2014-15, standing as the second largest producer of fruits and vegetables after China. India was able to export fruits worth Rs.4,448.08 crores/ 667.51 USD Millions and vegetables worth Rs.5,921.88 crores/ 884.75 USD Millions during 2016-17 (www.apeda.gov.in). Vegetables and fruits containing high levels of nutrient elements and sugars subject them to a range of diseases and disorders [18]. Fresh fruit and vegetables can be infected by pathogenic fungi and bacteria during crop growth in the field, harvesting, postharvest, storage and consumption. Numerous bacteria can also play spoil sport in storage. Approximately, 25 and 38% of harvested fruits and vegetables, respectively,

are lost to postharvest spoilage in the U.S. and global markets [4]. In India, generally, about 30% fruits and vegetables are rendered unfit for consumption due to spoilage after harvesting. Therefore, it is important not only to grow more, but also to save what is grown at high cost [90].

Postharvest diseases are caused primarily by microscopic bacteria and fungi where fungal pathogens are the main causal agent of fresh fruit and vegetable rot in postharvest processes [35]. Post-harvest pathogens perpetuate on crop debris in the field and under suitable conditions produce abundant spores. These fungal spores are easily carried by winds, rain, or dispersed by insects to flowers and young fruits at various stages of development and form a potential source of infection. Soil, irrigation water and infected plant debris forms an important source of infection. Soil-residing fungi and bacteria can attack the bulb, tuber, root and other vegetal parts through contact with the soil, lifting of soil particles by winds, rains or by arriving in storage with attached soil residues [9]. These pathogens secrete cellulose enzyme which degrade the tough cell wall of fruits and vegetables for infection to occur [63]. Post harvest pathogens can infect fruits and vegetables through two approaches. In 'quiescent' or 'latent' infections the pathogen can infect the host usually before harvest but the infection proceeds further after the host tissue turns conducive e.g. by the physiological changes during ripening. Anthracnose of various tropical fruits and grey mould of strawberries are caused by 'quiescent' or 'latent' infections by *Colletotrichum* spp. and *Botrytis cinerea* respectively. The other mode of infection is caused during and after harvest through injuries and wounds on the surface of crop produce. Blue mould disease caused by *Penicillium italicum* Wehmer, *Penicillium expansum* (Link) Thom and other *Penicillium* spp. in pome fruit, stone fruit, grapes, berries, citrus fruit, tomato, cucumber, melon and green mould caused by *Penicillium digitatum* Sacc. exclusively to citrus fruits etc. are such type of infections. Similar mode of infection is also adopted by *Rhizopus stolonifer* and other *Rhizopus* spp. causing rots in stone fruit, mango, grapes, berries, papaya, cucurbits, tomato, carrot, etc. Apart from these fungi several other species evokes serious implications on post harvest fruits causing specific symptoms. One of such fungal pathogens is *Alternaria alternate* causing fruit rot, sooty mould, dark spot where as *Alternaria citri* causes stem end rot. Black rot of tomato, melon and grapes is caused by *Aspergillus niger*. Crown rot symtoms could be developed by many fungi like *Acremonium spp., Ceratocystis paradoxa, Fusarium pallidoroseum, Verticillium thiobromae*. Several fungal species like *Monilinia fructicola, Monilinia fructigena, Phytophthora citrophthora* causes brown rot in many economically

important fruits. Watery soft rot in fruits and vegetables is manifested by *Mucor hiemalis, Mucor piriformis* and *Rhizopus stolonifier* where as watery white rot is caused by *Sclerotinia sclerotiorum*. Yeasty rot of tomato caused by *Geotrichum crrndidum* where as the same rot in pineapple is caused by pathogenic yeast belonging to *Saccharomyces* spp. However, bacterial species like *Ertuinia, Pseudomonas, Bacittus, Lactobacillus* and *Xanthomonas* are involved in post harvest soft rot of vegetables [9,23,101] (www.biologydiscussion.com).

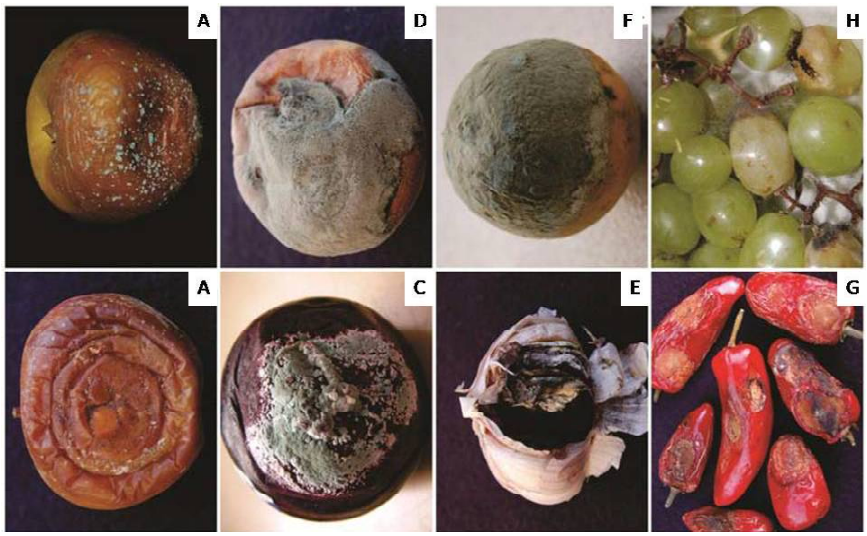

Figure-3: Examples of post-harvest diseases of fruits and vegetables. Bitter Rot (A) and blue mold (B), postharvest decay of apple caused by the fungus *Colletotrichum* spp. and *Penicillium expansum* respectively; (C) - Decay of nectarine fruitcaused by *P. expansum*; (D) - Brown Rot of peach caused by *Monilinia fructicola*; (E) - Black Mold caused by *Aspergillus niger* on garlic; (F) - Green mold caused by *P. digitatum* on citrus fruits; (G) - Anthracnose of pepper fruit caused by *Colletotrichum* sp.; (H) - Decay of table grapes caused by *Rhizopus stolonifer* and *Aspergillus niger:* **Courtsey: Gomes *et . al.*, 2015.**

1.7. Management of post harvest diseases of food produce

Post harvest diseases need to be controlled and managed to stop food loss. Exclusion of microbial pathogens at all stages before and after harvest is the basic and essential step to prevent access to the harvested produce. Deterioration of crops by microbial pathogens may be prevented by restricting them from entering into the crop produce from the very beginning stage i.e. seed treatment before sowing. Physical method of disease management include separation of infected materials, hot water treatment of seeds, application of heat in storage, sterilization by infrared, UV and gamma irradiation, microwave treatment, reduction of respiration

by applying wax etc. Chemical methods of control includes the application of an array of synthetic chemicals like Sodium and Potassium Carbonate and bicarbonate, Sodium orthophenylphenate, Ethanol and many other volatile compounds [71]. Other alternatives include the use of resistant crop cultivars. Most of the post harvest diseases are caused by fungi as discussed in the previous sections, hence an array of fungicides are currently being applied worldwide as a primary means of controlling postharvest diseases to prevent fungal contamination in crop produce [28]. The use of synthetic chemicals is continually increasing and is slowly restricted in many countries as a result of scientific concerns about their persistence in the environment and as well as adverse effect to human health. Further, resistant pests and pathogens are being evolved due to non-judicial and over application of fungicides which in turn puts an extra cost for developing new improved fungicides [117].

Because of the changing socioeconomic climate and recent advances in genetic engineering, interest in biological control as a meaningful approach to pest and disease management has been rejuvenated. Considerable interest has been given in the use of antagonistic microorganisms for the control of post-harvest diseases in recent years. The source of such organisms can be fermented food products, soil, plant tissues, surfaces of leaves, fruits and vegetables, etc. Once isolated, the microorganisms (bacteria, yeasts or filamentous fungi) can be screened in various ways for their efficacy to inhibit selected pathogens. As prevention is always better than cure, pathogen inhibition is effectively better and greater when the antagonist is applied prior to infection taking place [69,116]. In this connection the potential of endophytic microorganism have been bio-prospected for the last few decades. Endophytes are microbes (mainly bacteria and fungi) that colonize living, internal tissues of plants without causing any immediate, overt negative effects. Beneficial endophytic microorganisms comprise especially fungi and bacteria are plant associated microbes those form association with their host plants by colonizing the internal tissues without causing visible damage to their hosts, which has made them valuable for agriculture as a tool in improving crop performance [40,70]. Many endophytic microbes can have profound impacts on plant communities. They may increase fitness by conferring abiotic and biotic stress tolerance, increasing biomass and decreasing water consumption, or may decrease fitness by altering resource allocation [94]. Endophytic microorganisms have frequently been re-ported to be associated with crop plants, including wheat (*Triticum aestivum*), wild barley (*Hordeum brevisubulatum* and *Hordeum bogdanii*), soya bean (*Glycine*

max), and maize (*Zea mays*) and many tuber crops [74,75]. Some of the endophytic fungi in these crops conferred resistance of the plant to insect or fungal pathogens [121]. Endophytic fungi are known to be a rich source of novel antimicrobial substances. The suppression of plant diseases due to the action of endophytic microorganisms has been demonstrated in several pathosystems. Several mechanisms may control this suppression, either directly on the pathogen inside the plant by antibiosis and competition for nutrients or indirectly by induction of plant resistance response [40]. As endophytes live in a steady environment inside the plant, they have more antagonistic potentiality than microorganisms isolated from rhizosphere, plant surface, or soil [98]. Endophytic microbes which are potential sources of bioactive agents thus expected to be an effective, specific and eco-friendly approach to control post harvest disease causing pathogens especially in the scenario of post harvest environment and changing climate. The potential of many such endophytic microorganisms isolated from various sources to control post harvest pathogens has been discussed in the forthcoming sections.

1.8. Antagonistic Potential of Endophytes against Post harvest Pathogens

Discussions in the previous sections have brought out the association of number of microbial pathogens in post harvest duration with major cereals consumed throughout the world. Many of such organisms also produce notorious toxins which are harmful to human as well as to animals. Some of the plant pathogens might infect the crops before harvest and subsequently deteriorate the harvested produce in storage or post harvest interval. Hence, all sort of post harvest pathogens need to be addressed for inhibition. However, it is to accredit the effort of post harvest scientists and pathologists by whom many endophytic microorganisms have been isolated and their potential have been enumerated for the management of post harvest pathogens. Bacterial, fungal and even actinomycetes endophytes from internal tissue of various plant species have been found to possess antagonistic effects against post harvest pathogens. Table-1 gives a summary of some of such efficient endophytic microorganisms.

1.9. Endophytic fungal antagonist against post harvest pathogens

As an important measure to develop low toxicity, green agriculture is using antagonists to serve as biocontrol pesticide. Endophytic microorganisms have been pioneer in this context especially in inhibiting number of mycotoxigenic fungi. Even many *Aspergillus* species having endophytic life cycle could antagonize post harvest toxigenic *Aspergillus*

species and other toxigenic fungi. *Aspergillus terreus* and *Aspergillus flavipes* were such endophytes from medicinal plants *Achyranthus aspera* and *Stevia rebaudiana* respectively which could inhibit not only mycotoxigenic *Aspergillus* and *Fusarium* but also controlled the growth of other pathogens like *Sclerotinia, Alternaria, Bipolaris* etc. with more than 50% inhibition of mycelia growth. Screening of *Aspergillus terreus* revealed its antibacterial activity towards *S. aureus, A. hydrophila* and *E. faecalis* [39,113]. *Fusarium* app. might also behave as endophytes and many such species have been isolated from *Monarda citriodora*, an aromatic and medicinal plant. These endophytes could exhibit strong resistance to pathogens *F. solani* (59.6%), *Sclerotinia sp.* (100%), *Colletotricum capsici* (100%), *A. flavus* (98%) and *A. fumigatus* (100%) [40]. The two mycotoxigenic fungi *Aspergills flavus and Fusarium solani* had to face competitive inhibition for substrate by two antagonistic endophytes *Trichoderma viride and Botryosphaeria quercum* from healthy pods of cocoa where *B. quercum* showed highest Biocontrol index (BCI) of 63.3% and 59.7% respectively [114]. Some unique endophytes like *Paraconiothyrium variabile* isolated from *Cephalotaxus harringtonia* did not produce secondary metabolites having antagonistic effect to *F. oxysporum*. Instead it produced a competition-induced metabolite 13-oxo-9,11-octadecadienoic acid which could modulate the biosynthesis of beauvericin, one of the most potent mycotoxin of *F. oxysporum*, during the competition with the endophyte [24]. Similarly, many bioactive volatile organic compound (VOC) metabolites have been detected in endophytes *Nodulisporium* sp. strain GS4d2II1 (*Hypoxylon anthochroum*) [95] and *Hypoxylon anthochroum* strain Blaci from Leaves of *G. sepium* (Jacq.). Six VOCs' mixture (TM), alcohols' mixture (AM), phenylethyl alcohol (A), 2-methyl-1-butanol (2-M), 3-methyl-1-butanol (3-M), eucalyptol (E), ocimene (O), and terpinolene (T) at various concentrations could affect the respiration and cell membrane permeability of *F. oxysporum* growing on cherry tomatoes [65]. Similarly, a mixture of at least 28 volatile organic compounds, having very promising for post-harvest control, have been produced by the endophytic fungus *Daldinia concentrica*, isolated from an olive tree in Israel. These VOCs protected dried fruits of apricot, plum and raisin from rotting. Moreover, they protect peanuts against *Aspergillus niger*, oranges and tomato paste against *Penicillium digitatum* and grapes against *Botrytis cinerea*. Artificial mixtures of selected volatiles have great promise for application in food industry and agriculture [58]. *Penicillium* sp. are generally regarded as storage fungi having the ability to produce mycotoxins. However, some strains might act as endophytes like that of from wormwood roots (*Artemisia absinthium*) and could restrict *Botrytis*

cinerea to 50% growth in dual culture [78]. Some useful endophyte like *Phaeosphaeria nodorum* from 15 years old plum trees (*Prunus domestica* L.) in USA orchards, have also produced VOCs like ethyl acetate, 3-methyl-1-butanol, acetic acid, 2-propyn-1-ol and 2-propenenitrile which could inhibit growth and reduced width of the hyphae, and caused disintegration of the hyphal content of *Monilinia fructicola, Colletotrichum gloeosporioides* [85].

Fungal group belonging to *Muscodor* species has been recognised as producer of similar bioactive VOCs. One of this xylariaceaous fungi *Muscodor albus* was first isolated from small limbs of *Cinnamomum zeylanicum* (cinnamon tree). Each of the five classes of volatile compounds produced by the fungus (alcohols, esters, ketones, acids and lipids) had some inhibitory effect against post harvest pathogenic fungi like *Pythium ultimum, Phytophthora cinnamomi, Rhizoctonia solani, Ustilago hordei, Stagnospora nodorum, Sclerotinia sclerotiorum, Aspergillus fumigatus, Staphylococcus aureus, Bacillus spp.* [104]. Ramin *et. al*. (2005) found that 0.25g/L of dry VOCs of *M. albus* with air circulation might kill post harvest fungal pathogens. Major VOC by this fungus isobutyric acid (IBA) and 2-methyl 1-butanol (MB) could kill or suppress *Sclerotinia sclerotiorum*, *Botrytis* and *Penicillium expansum* at concentration of 40, 25, 45 µL/L and 75, 100 and 100 µL/L respectively. One minor VOC ethyl butyrate (EB) was only able to kill *Sclerotinia sclerotiorum* at 100ml/l. IBA and MB was also found to be effective against bacterial pathogens like *Erwinia carotovora pv. carotovora, Pseudomonas fluorescens, Escherichia coli*. Mercier and Jimenez (2004) fumigated apples for seven days with culture of *M. albus* grown on autoclaved grain which gave complete control of blue mold (*Penicillium expansum*) and gray mold (*Botrytis cinerea*) in wound-inoculated fruits. In wound-inoculated peaches, 24-72 h fumigation with *M. albus* provided complete control of brown rot (*Monilinia fructicola*). The major VOC 2-methyl-1-butanol and isobutyric acid were also effective against *Colletotrichum Geotrichum* and *Rhizopus*. Other VOCs 2-methylpropanoic acid and 3-methylbutan-1-ol produced by *Muscodor suthepensis* showed median effective doses (ED_{50}) on *Penicillium digitatum* growth of 74.91±0.73 and 250.29±0.29 5ØIÞLL”1 airspace respectively. Mycofumigation with a 30 g rye grain culture of *M. suthepensis* could control post harvest tangerine fruit rot [106].

Various *Trichoderma* species have traditionally been accepted as biocontrol agents of plant pathogens including many mycotoxigenic storage pathogens. *Trichoderma koningii*, an endophytic species of this fungal group from maize root was found to have *in vitro* and *in vivo* antagonism and

along with *Alternaria alternate* could grow on the mycelia of *F. verticilloides, F. oxysporum*, thereby reducing the radial growth by 25-75% and 53-80%, respectively [82]. In a dual culture assay *Trichoderma polysporum* from mountain-cultivated ginseng (*Panax ginseng* Meyer) showed significant inhibitory activity (45.6–78.6%) against mycelial growth of ginseng pathogens. Inhibitory effect was highest on the mycelial growth of *C. destructans*, with a reduction of 78.6% compared to the control whereas the same for *Pythium* sp., *Alternaria panax* and *Botrytis cinerea* was 55–70% inhibition, while the lowest inhibition was detected in *R. solani* (45.6%). *T. polysporum* was overgrown against *Pythium* sp., *A. panax* and *C. destructans* with profuse sporulation, which rapidly colonized the complete plate. Other fungal endophytes like *Tricharina ochroleuca, Lachnum virgineum, Phoma sp., Alternaria longissima, Penicillium chrysogenum* from the same source also had antagonistic effect towards pathogens [84].

1.10. Bacterial Endophytes: A major weapon against post harvest pathogens

Bacteria are having short generation period hence fast growing thus possess advantage over fungal antagonists to be used as bio-control agents. Many endophytic bacteria showed enormous potential to minimize the effect of post harvest pathogens especially mycotoxigenic and pathogenic *Fusarium* and *Aspergillus* species. *Fusarium oxysporum* has been the most hunted pathogen by many endophytic *Bacillus* bacteria. Mycelia of *F. oxysporum* has been inhibited up to 43% by *Bacillus* species from black pepper roots [29]. Glassner *et. al*., 2015 isolated number of endophytic bacteria like *Bacillus amyloliquefaciens, Bacillus pumilus, Bacillus koreensis, Bacillus brevis, Bacillus subtilis, Streptomyces sp, Sphingomonas sp., Bacillus vallismortis, Bacillus megaterium, Bacillus safensis* from fruits of *Cucumis melo Reticulatus* Group 'Dulce'. These antagonists could inhibit number of *F. oxysporum* species and pathotypes like *melonis races and radicis-cucumerinum,* possessing potential to control further post harvest damage [36]. Three Egyptian bacterial endophytes *Stenotrophomonas maltophilia, Bacillus subtilis and Pseudomonas aeruginosa* from healthy tissues of *Brassica oleracea* (Root), *Capsicum annuum* (Stem) and *Pisum sativum* (Root) exhibited sharp in vitro antagonistic effect by forming inhibition zones against *F. solani* (29 mm, 37 mm and 41 mm respectively), against *R. solani* (43mm, 39mm and 35mm respectively), against *Pythium ultimum* (39mm, 27mm and 35mm respectively), against *Erwinia carotovora* (25mm, 45mm and 49mm respectively). Aflatoxigenic fungus *Aspergillus flavus* could not be inhibited

by *S. maltophilia* in this study [98] but 100% inhibition of *A. flavus* could be observed by cotton endophyte *Pseudomonas cepacia* in both *in vitro* as well as in field applications [67].

A. Endophytic *Pseudomonas sp*.

Other than *P. cepacia* many other species of this genus in general have been known for their antagonistic activity against a wide range of agricultural pathogens. *Pseudomonas cepacia* isolated as endophyte of papaya pericarp was able to colonise in the lamina, leafstalk, pericarp and pulp of papaya and strongly exhibited broad spectrum anti pathogenic activity against *Phytophthora nicotianae, Peronophythora litchi, Erwinia carotovora, Phytophthora capsici, Colletotrichum gloeosporioides, Colletotrichum higginsianum, Alternaria tenuis, Fusarium oxysporum, Penicillium italicum, Rhizopus stolonifer*. The application of the antagonist at five preharvest stages of papaya significantly reduced the disease index of anthracnose, with the best control effect reaching 63% after application at the florescence stage [99]. Similarly *Pseudomonas fluorescens* isolated from bean inhibited the growth of *Rhizoctonia solani, Pythium ultimum, Sclerotium rolfsii*. Some *Bacillus* sp. endophytes found in this study by Pleban *et. al*., (1995) were inoculated in to cotton plant to study in vivo efficiency. *B. cereus*, *B. subtilis* and *B. pumilus* reduced 51%, 46% and 56% disease incidence by *Rhizoctonia solaniI* and 79%, 72% and 26% disease incidence by *Sclerotium rolfsii*. Pleban *et. al*., (1995) did not use *Pseudomonas* for this purpose. However, Abla *et. al*., (2015) isolated 59 bacterial strains from roots of *Mentha rotundifolia* L. mostly belonging to Pseudomonadaceae family. In addition to the antagonism to *F. oxysporum*, the endophytes could also restrict *Aspergillus Niger and Botrytis cinerea* with inhibition of more than 60%.

***B. Bacillus* endophytes**

Bacteria belonging to *Bacillus* species are common antagonists as they form the bacterial group which produces large number of secondary metabolites including antibiotics and several extracellular enzymes. The bioactivity was observed to be even better when the life style turns endophytic in many cases. Endophytic species like *B. megaterium* and *Bacillus cereus* showed profound antagonistic effect on post harvest pathogens. Ramnujam *et. al*., (2012) screened 83 endophytic bacterial strains isolated from chilly fruits which suppressed the lesions of anthraconose disease caused by *Colletotrichum capsici* (Syd.), *B. megaterium* being the most efficient with 60% inhibition. Cheng *et. al*., (2015) developed a modified formulation for field application which

included 60% *B. cereus* freeze-dried powder, 28.9% diatomite as carrier, 4% sodium lignin sulfonate as disperser, 6% alkyl naphthalene sulfonate as wetting agent, 1% K_2HPO_4 as stabilizer, 0.1% â-cyclodextrin as ultraviolet protectant. The endophytic *B. cereus* had been isolated from chest nut seed and in long storage life it retained efficacy to control *Endothia parasitica* (Murr) and *Fusarium solani* which caused fruit rot in chestnut and other fruis. Apart from this, crude methanolic extract of lipopeptides from *Bacillus amyloliquefaciens* strain TF28 was found disintegrating the hyphae and spore of mycotoxigenic fungus *F. moniliforme*. The extract could retain its antifungal activity even after treatment at pH values ranging from 2 to 12 for 24 h or at 100°C for 30 min [122]. Bacon *et. al*., (2001) analysed 13 strains of *B. mojavensis,* isolated from major deserts of the world, endophytically colonized maize and were antagonists to *F. moniliforme*. The endophytic colonization of maize by *B. subtilis* and other species within this subgroup of the Bacillaceae varied, as did antagonism, to *F. moniliforme*. As discussed previously *Botrytis cinerea* causes gray mould in fruits and vegetables could be controlled by the application of several endophytic bacterial antagonists. *Brevibacillus brevis* W4 isolated from healthy tomato leaves and stems exhibited inhibition rate of 78% in dual culture assay and 100% when 20 times diluted fermented filtrate was used against *Botrytis cinerea*. The filtrate could resist UV radiation [119]. Biological Control of Gray Mold in Pears caused by this pathogen could be achieved best when the bacterial antagonists mixed with the fungicide at 50 ppm a.i. than single treatment. Holding bacteria-treated pear fruits at 20°C for 24 h before cold storage improved the efficacy of the bacteria against gray mold [61]. Similarly number of bacterial isolates like *B. subtilis, B. brevis, B. amyloliquefaciens, B. azotoformans, B. licheniformis* could be isolated from stored apples which reduced blue mold decay when apples were stored at 5, 10, and 20°C and gray mold decay significantly reduced at 5 and 10°C [100]. *Botrytis cinerea* and *Monilinia laxa* have been the causal postharvest rots of sweet cherries and table grapes. *Monilinia laxa* which is otherwise known as 'blossom blight' causes brown rot of stone fruits. An atypical bacterial endophyte *Aureobasidium pullulans* from these fruits significantly reduced pathogen load on table grape berries when applied 6, 12, and 24 h after inoculation. Reduction of rots was in the range of 32 to 80% (sweet cherries) and from 59 to 64% (table grape) when the antagonist applied after harvest. *Aureobasidium pullulans* strains could survive in field conditions as well as cold storage and also able to penetrate the flesh of sweet cherries when applied during flowering [96]. Around 122 bacterial endophytes isolated by Pratella *et. al*., (1993) from sub epidermis of cucumber, eggplant, pepper, tomato,

zucchini, apricot, peach and plum plants were tested against *Monilinia laxa and Rhizopus stolonifer* causing post harvest rots in fruits and vegetables. Twenty isolates could reduce 90% rot incidence where inhibition of *R. stolonifier* was less except one strain. Most of the tested isolates in this study had only a temporary protective action; delaying the spread of infection still several endophytic bacteria afforded complete protection against *M. laxa* for up to 6 days at 20°C.

C. Endophytic *Actinomycetes*

Though the potential of Actinomycetes have been efficient antagonists to many phytopathogenic fungi and bacteria but their potential to control post-harvest diseases of fruits and vegetables is least explored. Still some endophytic actinomycetes exhibited antagonism to many post harvest pathogens. Number of such strains belonging to *Streptomyces* sp. have been isolated from healthy maize plants in Sao Paulo and inhibited pathogens like *Pythium aphanidermatum, Rhizoctonia solani, Sclerotinia sclerotiorum, Phytophthora parasitica, Fusarium sp.* Even under green house experimentation some of these isolates could reduce damping off disease up to 71% [25]. Cao *et. al*., (2005) isolated 131 endophytic actinomycete strains belonging to *Streptomyces*, *Streptoverticillium* and *Streptosporangium* species from surface-sterilized banana roots. More than 18% strains were having inhibitory effect to *F. oxysporum* f. sp. *cubense*, the pathogen of Panama disease. The pathogen also exhibited mycotoxigenic effect previously. The antibiosis assay on autoclaved banana pseudostem tissue showed that the distribution of mycelia of this pathogen decreased in one site near the colony of endophytic *Streptomyces* strain in banana tissue.

1.11. Post Harvest Disease Control by Endophytes: The way forward

The preceding discussion has brought out several features of endophytic microorganisms to be used as potential biocontrol agent to minimize or stop post harvest diseases of fruits, vegetables and also in cereal grains. Post harvest pathogens including mycotoxin producing fungi have shown susceptibility to these antagonists. However, like many of other similar research outcomes, these antagonists are still to be utilized under field applications and their performance under large-scale and commercial ventures are still to be established firmly. Few reports of course described regarding the preparation of bio-formulations comprising endophytic microorganisms but the feasibility and their utilization by farmers at grass root level must be taken care of. Even the vast number of isolated endophytes has not been subjected to genetic improvement by advanced

technology like mutation by radiation or by chemical mutagens like DMSO. As observed in many endophytic researches, suitable inoculation methods also need to be developed for the establishment of the endophytic antagonists in the plant of interest. Mycofumigation process needs further precision and the effectiveness need to be further extended. Application of these endophytic antagonists in storage conditions to control mycotoxigenic fungi still remains a big challenge for post harvest researchers. Volatile Organic Compounds produced by many endophytic antagonists have very useful in this context but the current scenario of post harvest storage needs further research for the development of a commercially viable product which must be eco-friendly in nature. As indicated by many researchers in this particular subject, using endophytic or any microbial antagonists as "living fungicides" on food materials holds a special problem as the microbial cells, fragments and metabolites are supposed to be consumed by humans and animals. Such exposure might lead to development of antimicrobial resistance of human and animal pathogens. Though microorganisms had a history of being used as food preservers, still possible pathogenicity to man and other animals, as well as a wide range of harvested commodities must be considered when such use is being thought of.

However, the first step towards all the above future research should have begun with highlighting the enormous potential of fungal, bacterial even actinomycetes endophytes to inhibit or restrict the post harvest pathogens. The current review may be considered as a small but significant stepping stone towards this horizon. This review may act as a supplement to pathologist and post harvest scientists to devise long term and sustainable approach involving the endophytic antagonists for the best management of post harvest loss of food crops and post harvest pathogens.

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Table-1: Antagonistic effect of endophytic microorganisms (Fungi, Bacteria and Actinomycetes) against post harvest pathogens of fruits, vegetables and cereal grains.

Endophytic microorganism	Source of isolation	Post harvest pathogens against which antagonism observed	References
Aspergillus terreus	*Achyranthus aspera*, (medicinal plant)	*Alternaria alternate, Fusarium oxysporum, Bipolaris soronkiniana, Aspergilus flavus, Phytopthora sp, S. aureus, E. faecalis, A. hydrophila*	[39]
Aspergillus flavipes	foliar tissues of medicinal plant *Stevia rebaudiana* Berton	*Sclerotinia sclerotiorum*	[113]
Trichoderma koningii, Alternaria alternate	Maize root	*F. verticilloides, F. oxysporum*	[82]
Meliniomyces variabilis, Cadophora sp.	Barley, Chinese cabbage, eggplant	*F. oxysporum*	[47]
Penicillium sp.and unidentified fungal strain B9C22	wormwood roots (*Artemisia absinthium*)	*Botrytis cinerea*	[78]
Phaeosphaeria nodorum	plum (*Prunus domestica*) leaves	*Monilinia fructicola, Colletotrichum gloeosporioides*	[85]
Tricharina ochroleuca, Trichoderma polysporum, Lachnum virgineum, Phoma sp., Alternaria longissima, Fungal endophyte sp., Penicillium chrysogenum	mountain-cultivated ginseng (*Panax ginseng* Meyer)	*Botrytis cinerea, Rhizoctonia solani, Alternaria panax, Cylindrocarpon destruntans Pythium sp*	[84]

Fusarium oxysporum, F. oxysporum, F. oxysporum F. redolens, Muscodor yucatanensis	*Monarda citriodora* Cerv. ex Lag. (Lamiaceae/Labiatae)	*F. solani, Sclerotinia sp., Colletotricum capsici, A. flavus, A. fumigatus*	[46]
Trichoderma viride and Botryosphaeria quercum	Cocoa *(Theobroma cacao L.)*	*Aspergills flavus and Fusarium solani*	[114]
Paraconiothyrium Variabile	needle of *Cephalotaxus harringtonia* var. *drupacea*	*Fusarium oxysporum*	[24]
Muscodor albus	small limbs of *Cinnamomum zeylanicum* (cinnamon tree)	*Pythium ultimum, Phytophthora cinnamomi, Rhizoctonia solani, Ustilago hordei, Stagnospora nodorum, Sclerotinia sclerotiorum, Aspergillus fumigatus, Staphylococcus aureus, Bacillus spp.Botrytis cinerea, Penicillium expansum, Sclerotinia sclerotiorum, Erwinia carotovora pv. carotovora, Pseudomonas fluorescens, Escherichia coliBotrytis, Colletotrichum, Geotrichum, Monilinia, Penicillium and Rhizopus.*	[104] [89] [66]
Muscodor suthepensis	*Cinnamomum bejolghota*	*Penicillium digitatum*	[106]
Daldinia concentrica	Olive tree	*Aspergillus niger, Penicillium digitatum, Botrytis cinerea*	[58]
Nodulisporium sp. strain GS4d2II1 (Hypoxylon anthochroum)	Leaves of *G. sepium* (Jacq.) Kunth exWalp. (1842)	*Pythium aphanidermatumFusarium oxysporum*	[65,95]
Actinomycetes *(Streptomyces spp.)*	Maize (roots, leaves, stems)	*Pythium aphanidermatum, Rhizoctonia*	[25]

		solani, Sclerotinia sclerotiorum, Phytophthora parasitica, Fusarium sp.	
Streptomyces, Streptoverticillium and *Streptosporangium*	Banana roots	*Fusarium oxysporum f. sp. cubense*	[17]
Stenotrophomonas maltophilia, Bacillus subtilis, Pseudomonas aeruginosa	*Brassica oleracea* (Root), *Capsicum annuum* (Stem), *Pisum sativum* (Root)	*Fusarium solani, Fusarium oxysporum, Pythium ultimum, Sclerotium rolfsii, Aspergillus flavus, Aspergillus niger, Rhizoctonia solani, Alternaria solani, Erwinia carotovora, Erwinia carotovora*	[98]
Bacillus amyloliquefaciens TF28	soybean root	*Fusarium moniliforme*	[122]
Bacillus spp.	Black pepper roots	*F. oxysporum*	[29]
Bacillus amyloliquefaciens, Bacillus pumilus, Bacillus koreensis, Bacillus brevis, Bacillus subtilis, Streptomyces sp, Sphingomonas sp., Bacillus vallismortis, Bacillus megaterium, Bacillus safensis, Massilia sp.	fruits of *Cucumis melo Reticulatus* Group 'Dulce'	*Macrophomina phaseolina, Fusarium oxysporum f. sp. melonis races 1 and 2, F. oxysporum f. sp. radicis-cucumerinum (Forc), Pseudomonas syringae (P.s.).*	[36]
B. mojavensis, Bacillus subtilis RRC101	Maize seedling roots	*Fusarium moniliforme/ F. verticilloides*	[8]
Bacillus, Pseudomonas spp.	*Mentha rotundifolia* L.	*Fusarium oxysporum, Aspergillus Niger and Botrytis cinerea*	[2]
Aureobasidium pullulans	flesh of sweet cherries	*Botrytis cinerea* and *Monilinia laxa*	[96]

Bacterial Endophytes	cucumber, eggplant, pepper, tomato, zucchini, apricot, peach and plum	*Monilinia laxa and Rhizopus stolonifer*	[87]
B. subtilis, B. brevis, B. amyloliquefaciens, B. azotoformans, B. licheniformis	Stores apple	*Penicillium expansum, Botrytis cinerea*	[100]
Bacillus pumilus, Bacillus amyloliquefaciens	Skin of fruits and vegetables	*Botrytis cinerea*	[61]
Brevibacillus brevis	Tomato leaves and stems	*Botrytis cinerea*	[119]
Pseudomonas fluorescens, Bacillus cereus, Bacillus subtilis, B. cereus, B. pumilus	Bean, *Sinapis*, Onion, cauliflower, sunflower	*Rhizoctonia solani, Pythium ultimum, Sclerotium rolfsii*	[86]
B. megaterium,	Leaves/fruits tissue of Chilli	*Colletotrichum capsici*	[88]
Bacillus cereus	chestnut fruit	*Endothia parasitica* (Murr) *and . Fusarium solani*	[20]
Pseudomonas putida	pericarp of papaya	*Phytophthora nicotianae, Peronophythora litchi, Erwinia carotovora, Phytophthora capsici, Colletotrichum gloeosporioides, Colletotrichum higginsianum, Alternaria tenuis, Fusarium oxysporum, Penicillium italicum, Rhizopus stolonifer*	[99]
Pseudomonas cepacia	stems, unopened flowers, cotton boll surfaces.	*Aspergillus flavus*	[67]

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