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Application of various bioagents for the management of post-harvest of horticultural crops

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Abstract: Post-harvest diseases cause considerable losses to harvested fruits and vegetables during transportation and storage. Synthetic fungicides are primarily used to control post-harvest decay loss. Some of these pathogens are: Fusarium oxysporum, Ralstonia solanacearum, Pythium spp., Phytophthora spp, Xanthomonas spp., Colletotrichum spp., Pencillium spp , Alternaria spp etc. Many soil-borne diseases as well as post-harvest fungi are effectively addressed through a variety of bioagents belonging to predominant microbial genera represented by Trichoderma spp., Bacillus spp., Gliocadium spp. and fluorescent Pseudomonads. Of various biological approaches, the use of antagonist microorganisms is becoming popular throughout the world. Several post-harvest diseases can now be controlled by microbial antagonists. Plant-based products can be utilized at post-harvest stage washing agents, botanical sanitizers, edible coatings (protein, lipid and starch-based, anti-microbial agents, cushioning materials during transport, preservatives and as control measure for storage diseases as various bioactive constituents. In addition, production of antibiotics, direct parasitism, and possibly induced resistance in the harvested commodity are other modes of their actions by which they suppress the activity of post-harvest pathogens in fruits and vegetables. To overcome this problem and in order to take the bio-control technology to the field and improve the commercialization of bio-control, it is important to develop new formulations of bio-control microorganisms with higher degree of stability and survival. It is possible to use the biological control as an effective strategy to manage plant diseases, increase yield, protect the environment and biological resources and approach a sustainable agricultural system.

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Key words: Post-harvest diseases, antagonist microorganisms, bio-control technology

Introduction

Horticultural crops have an important place in the agricultural economy and a wide variety of fruits and vegetables has been produced in Pakistan with total annual production estimated at 13.72 million tons that include 6.67 million tons of fruits, 6.5 million tons of vegetables and 2.27 million tons of condiments. Among fruits, citrus is leading in term of production followed by mango, dates and guava. Potato and onion are leading among vegetables and condiments (GOP, 2012-13). Exports of fruits and vegetables from Pakistan stood at US$ 538 million in 2011-12 went up to US$ 625 during 2012-13. During July, 2012 –November, 2013 exports of vegetables were 109,378 tonnes worth of $47.4 million that increased to 197,855 worth of $63 million in 2013-14. During the same period export of fruits were 191,739 tonnes worth of $103 million that rose to. 218,203 tonnes worth of $143.4 million. The export of fruit & vegetable preparations (mostly juices) is US$ 5.6 million (4.2%). Their share in Pakistan's total exports is slightly over one percent (Abdullah, 2010; MINFSR 2013-14). Owing to lack of proper fruit processing industry and non-availability of post-harvest operations agriculture sector faces loss of worth Rs. 32-40 billion annually as up to 35 percent of the total agriculture produces face physical loss at various stages, after the crops have been matured and before the food is consumed (Sabir, 2009). Perishable fruits and vegetables (fruits and vegetables with more water content) facilitate the easy attack of the micro-organism due to high water activity and spoiled rapidly. Improper handling, storage, preservation techniques and microorganism spoilage increase the postharvest losses in fruits and vegetables up to 40%. The microbial effect plays a vital role in spoilage of fruits and vegetables due to some extensive heat or cold resistance microorganism the processed or canned product also can be damage (Sharma et al., 2013). Practices of postharvest technologies can reduce the quantitative and qualitative losses of fresh fruits and vegetables and also maintained the product quality up to final consumption. Attaining the hygienic agricultural produce should be focused on the varieties of higher post-harvest longevity (Wasala et al., 2014). Post-harvest loss of food commodities is a global challenge and it has been estimated that nearly 30%–50% of the fresh products were lost during the post-harvest storage due to pathogenic attack, improper storage, transportation, or packaging. The pathogenic attack during post-harvest storage is considered as the prime factor for post-harvest loss. Conventionally, breeders frequently utilized chemical pesticides or fungicides to manage pathogenic attack and enhance the shelf life of food products. However, inappropriate and continuous use of chemical pesticides lead to adverse impacts such as chemical residue, low-quality product, and environmental contaminants, or phyto-pathogens resistance. Thus, there is a need to search for an alternative, that must be eco-friendly, cost-effective, and free from chemical residue. The microbial antagonism is an emerging approach and is broadly utilized in managing the growth of pathogens during pre- or post-harvest storage.

Factors of post-harvest diseases

Commodity type, Cultivar susceptibility to postharvest disease, the postharvest environment (temperature, relative humidity, atmosphere composition, etc), Produce maturity and ripeness stage, Treatments used for disease control, Produce handling methods, Postharvest hygiene.

Post-harvest diseases

It is well known that, after harvesting, the quality of fruit and vegetables has decreased rapidly and many nutrients have been lost, particularly if the product is not effectively cooled (Duan et al., 2020). Every year, diseases cause post-harvest losses (40–60%) in vegetable crops due to their perishable nature under field (15–20%), packaging and storage (15–20%), and transport (30–40%). Profiling, detection, and diagnosis of postharvest vegetable pathogens (diseases) are essential for better understanding of pathogen and formulation of safe management of post-harvest spoilage of vegetables. The vegetable produce is spoiled by post-harvest pathogens and makes them unfit for human consumption and market due to the production of mycotoxins and other potential human health risks.

Fungal diseases

Genera of fungal pathogens viz. Alternaria, Aschochyta, Colletotrichum, Didymella, Phoma, Phytophthora, Pythium, Rhizoctonia, Sclerotinia, Sclerotium, and bacterial pathogens viz. Erwinia spp., Pseudomonas spp., Ralstonia solanacearum, Xanthomonas euvesictoria were recorded as post-harvest pathogens on vegetable crops.

Sclerotinia

White mold (Sclerotinia sclerotiorum) appears in warm and moist weather (>95% relative humidity) and favors fungal growth on infected pods which develops as a white, fluffy mycelial mat often with large, irregular, black-colored sclerotia, typical of S. sclerotiorum. Within the superficial mycelium, initially white but later hard dark black sclerotia are formed. Infected pods show brown discoloration and soft rot. The isolated fungus was identified as S. sclerotiorum based on morphological and cultural characteristics of the mycelia and sclerotia.Typical symptoms of Sclerotinia white rot in Indian bean, French bean, pea, brinjal, tomato, bottle gourd.

Ascochyta

Ascochyta blight (Ascochyta pisi) black spot symptoms on pods result in the production of round tan-colored sunken spots bearing dark margins. Pycnidia develop in the centers of such spots on pods, Mostly cause disease in leguminous vegetable crops.

Phytophthora

Initial disease symptoms appeared in the form of irregular; water-soaked and light brown lesions on leaves which are normally covered with white cottony mycelial growth on the lower side of leaves. Water-soaked brown lesions expanded rapidly on stem, green fruits and vegetables i.e Tomato (Solanum lycopersicum, solanaceae).

Alternaria

Different species of Alternaria cause rot of many fresh fruits and vegetables, e.g., black rot of orange, tuber rot of potato, sweet rot of sweet potato, purple blotch of onion, Alternaria rot of onion, Alternaria rot of cabbage, etc.

Botrytis

It causes grey mold rots of fruits like pear and citrus etc., and vegetables like onion, tomato etc. Every year it causes great economic loss.

Fusarium

It causes different diseases, commonly called pink or yellow molds. Different species of Fusarium cause damage to tubers, bulbs, storage roots etc and frequently cucurbits etc. It also causes brown rot of fruits like lemon, orange etc.

Penicillium

Species of Penicillium are commonly called blue or green molds, these cause rots of different fruits like onion, sweet potato etc. They also cause spots on different fruits. Under storage, the spotted fruits bear tufts of spores. Though most of the Penicillium species prefer relatively high temperature for their growth in storage, they still remain active near freezing temperature at a slow rate. A few species produce ethylene which increase respiration of fruits, thereby it reduces the storage life of the fruits. It also produces patulin, a mycotoxin which directly contaminates the sauces and fruit juices prepared from infected partly rotten fruits.

Bacterial diseases

Phytopathogenic bacteria cause post-harvest diseases of economically important vegetables. Different species of bacteria belonging to top ten genera viz. Pseudomonas; Ralstonia; Agrobacterium; Xanthomonas; Xanthomonas; Xanthomonas; Erwinia; Xylella; Dickeya (dadantii and solani); Pectobacterium are devastating plant pathogens (Pectobacterium soft rot on cauliflower). They are unable to penetrate directly into plant tissue; however, they enter through wounds or natural plant openings. Wounds can be caused by insects and tools during operations like pruning and picking of the produce. The bacteria only become more active and cause infection when factors are conducive. Factors conducive to infection are high humidity, crowding, poor air circulation, plant stress caused by over watering, under watering, or irregular watering, poor soil health, and deficient or excess nutrients. The bacteria multiply quickly when free moisture and moderate temperatures are available. The major causal agents of bacterial soft rots are various species of Erwinia, Pseudomonas, Bacillus, Lactobacillus, and Xanthomonas. Psuedomonas syringae pv. syringae, P. syringae pv. pisi and P. syringae pv. Phaseolicola causes diseases in vegetables. The symptoms appear as water-soaked spots on pods that become sunken and dark-brown in color with distinctive reddish-brown margins.

Management of Post Harvest Diseases and post-harvest losses of Vegetable, fruits and Crops

Post-harvest losses in vegetables are found due to fungal and bacterial infection worldwide. New challenges are faced under trade liberalization and globalization, and serious efforts are needed to reduce these losses in vegetables.

The effective bioagents viz., Trichoderma, Pseudomonas, Bacillus and Yeast screened under in vitro conditions were used for fruit dip and challenge inoculation method to evaluate their effectiveness against anthracnose disease of mango caused by Colletotrichum gloeosporioides. Most of the papaya postharvest loss usually occurs during the postharvest chain, so papaya had become the ideal target of various pathogens due to the fruit's structure, causing its market acceptability and shelf life to be affected in a negative manner (Sivakumar and Wall, 2013; Prasad and Paul, 2021). The impact of post-harvest pre-storage single exposure on most common post-harvest fungal pathogens, including Rhizopus, Penicillum digitatum, P. expansum and Penicillum italicum, Monilinia sp., Botrytis cinerea, Colletrichcum sp. and Fusarium sp. among others, with positive effects with regards to reducing disease incidence and severity. Direct germicide action compromising microbial viability has been frequently reported, but less severe effects, such as the reduced germination speed of viable conidia, have also been observed. With regards to human pathogens, the direct UV irradiation of fresh produce reduced the viability of E. coli, Salmonella, and Listeria. These studies have so far mostly been conducted on a laboratory or, in the best scenario, pilot scale. A review of the available research suggests that their widespread commercial use requires some important aspects to be solved, especially with regards to the adaptability of this treatment to continuous processing lines (where the treatment duration may require minutes at low irradiances), safety procedures for workers and even radiation exposure, while avoiding mechanical damage, especially to fruits and vegetables. Furthermore, the fact that wet cleaning methods have long been applied to fresh produce could limit the fast adoption of a different technology.

Biological control

International markets reject produce containing unauthorized pesticides, with pesticide residues exceeding permissible limits, and with inadequate labeling and packaging. Hence, biological control of postharvest diseases has great potential because postharvest environmental conditions like temperature and humidity can be strictly controlled to suit the needs of the biocontrol agent. Much information has been provided in relation to postharvest biocontrol and the problems faced by the development of commercial products. Biological control is used through microbes such as fungi, bacteria, actinomycetes, and viruses (bacteriophages) to control the postharvest disease of vegetables. The degree of disease control or disease suppression achieved with these bioagents can be comparable to that achieved with chemicals. As per estimates, the market of Indian bioagents is equivalent to 2.89% of the overall pesticide market in India with the worth of rupees 690 crores. It is expected to show an annual growth rate of about 2.3% in the coming years. In India, so far only 18 types of bio-pesticides have been registered under the Insecticide Act of 1968. Among agriculturally important microbes, Trichoderma viride, T. harzianum, Pseudomonas fluorescens, and Bacillus subtilis are the most potential bio-agents which as act as producers of biologically active metabolites like antibiotics and bacteriocin, elicitors and inducers of systemic resistance in plants. Biocontrol mediated pathogen inhibition is found to be more effective when the antagonist is applied prior to infection taking place. Antagonists which act against postharvest pathogens of vegetables by competitive inhibition at wound sites include the yeasts Pichia and Debaryomces species. Chitosan, for example, is not only an elicitor of host defense responses but also has direct fungicidal action against a range of postharvest pathogens. Trichoderma has potent antifungal activity against Botrytis cinerea, S. sclerotiorum, Cortictum rolfsi, and other important biotic stresses. Microbial pesticide active ingredients of Streptomyces griseoviridis K61 against bacterial soft rot, gray mold, Phytophthora; Gliocladium catenulatum against gray mold; Candida oleophila strain against postharvest diseases; Coniothyrium minitans against Sclerotinia sclerotiorum, Sclerotinia minor; Trichoderma aspellerum (formerly T. harzianum) against Pythium, Phytophthera, Botrytis, Rhizoctonia; Trichoderma atroviridae against B. cinerea and B. subtilis against Botrytis spp. is the most commonly used biocontrol agents for postharvest diseases.

Chemical control

Chemical fungicides are commonly used for the management of postharvest disease in vegetables. For postharvest pathogens which infect produce before harvest, the fungicides should be applied at field level during the crop season, and/or strategically applied as systemic fungicides. At the postharvest level, the fungicides are often applied to reduce infections already established in the surface tissues of produce or they may protect against infections occurring during storage and handling. Fungicides used during postharvest are actually fungistatic rather than fungicidal under normal usage. The fungicides are applied on the produce as dips, sprays, fumigants, treated wraps, and box liners or in waxes and coatings. Dip and spray methods are very common in postharvest treatments. The fungicides generally applied as a dip or spray method are benzimidazoles (e.g. benomyl and thiabendazole) against anthracnose, and triazoles (e.g. prochloraz and imazalil) and fumigants, such as sulfur dioxide, for the control of gray mold used for postharvest disease control. Dipping in hot water (at 50°C for 5–10 min, depending on the size of produce in combination with the fungicide) is also used for effective control of the disease. Sodium hypochlorite as a disinfectant is used to kill spores of pathogens present on the surface of the vegetable produce.

Table 1. Common post harvest diseases in fruits and vegetables

Commodity	Disease	Pathogen
Apple	Pencillium rot	*Pencilliumexpansum*
Strawberry	Graymold	*Botrytis cinerea*
Banana	Crown rot	*ColletotrichumMusae*
Tomato	Rhizopus rot	*Rhizopus nigricans*
Chilli	Anthracnose	*Colletotrichum capsica*
Grape	Graymold	*Botrytis cinerea*
Pear	Rhizopus rot	*Rhizopus stolonifera*
Tomato	Alternaria rot	*Alternaria alternate*

Table 2. Post harvest losses of fruits and vegetables

Region and country	Commodity	Losses (%)
Kenya	Banana	11.2-50
Tanzania	Sweet potato	32.5-35.8
Cambodia Loas	Yard-longbean	12.2-21.8
Loas Vietnam	Chilli pepper	10.7-16.9
Bangladesh	Litchi	8.0
Pakistan	Tomato PotatoOnion	20 12 9.0
Srilanka	Banana	20
Iran	Grapes	13
Ghana	Tomato	20
Egypt	Oranges Tomatoes	14 15
Egypt	Pomegranate Onion	23 19
Jordan	Tomato EggplantPepper Squash	18 19.4 23 21.9
Srilanka	Tomato	54
Saudi Arabia	Tomato CucumberFigs GrapesDates	17 21.3 19.8 15.9-22.8 15
Cambodia Loas Vietnam	Tomato	24.6 16.9 19.1
Nigera	Tomato Bell pepper Hot pepper	20-28 12-15 8-10

Table 3. Post harvest losses of fruits and vegetables in India

Fruits and Vegetables	Losses (%)
Mango	20-26
Banana	18.3-28.8
Grapes	14.4-21.3
Pomegranate	35.4
Potato	10.5-19.8
Tomato	11-35
Bell Pepper	6.7-17.1
Cabbage	9.4-30.4
Onion	12-30
Cucurbits	52
Cauliflower	12.9-35.1
Citrus	27
Litchi	30
Okra	31
Guava	20

Table 4. Microbial antagonists used for the successful control of post-harvest diseases of fruits and vegetables

Antagonists	Disease(pathogen)	Fruits/Vegetables
Candida olephila	Penicillium rot (*Penicilliumexpansum*)	Apple
Bacillus subtillis	Graymold (*Botrytis cinerea*)	Strawberry
Pichiaguillermondii	Crown rot (*Colletotrichummusae*) Rhizopus rot (*Rhizopus nigricans*)	Banana Tomato
Metschnikowia Pulcherrima	Blue mold (*Penicilliumexpansum* Graymold (*Botrytis cinerea*)	Apple
Candida olephila	Graymold (*Botrytis cinerea*)	Tomato
Pichiaguillermondii	Alternaria rot (*Alternariaalternata*)	Tomato
Trichodermaharzianum	Graymold (*Botrytis cinerea*)	Pear
Cryptococcus laurentii	Brown rot (*Monilinafructicola*)	Cherry
Pichiaguillermondii	Anthracnose (*Colletotrichumcapsici* (Syd.) Butler &Bisby	Chillies

Table 5. Commercially available bioproducts for control of post harvest diseases

S.No	Microorganism	Product name	Target pathogens	Fruit	Country
1.	*Aureobasidium pullulans*	Boniproduct	*Penicillium* *Botrytis* *Monilinia*	Pome fruit	Europe
2.	*Bacillus subtilis*	Avogreen	*Cercospora* *Colletotrichum*	Avocado	South Africa
3.	*Candida olephile*	Nexy	*Botrytis* *Penicillium*	Pome fruit	Belgium, EU
4.	*Pseudomonas syringae*	Biosave	*Penicilliu mBotrytis* *Mucor*	Pome Citrusfruit Cherry Potato Sweet potato	United States
5.	*Trichodermaharzianum T-39*	Trichodex	*Botrytis cinerea*	Most of food crops	Bioworks,USA

Postharvest handling operations of vegetable crops

Maintenance of hygiene in all stages of postharvest handling is critical to minimize the source of primary inoculum for postharvest diseases [39]. Produce should be harvested during the day instead of early morning. Field containers should be smoothed. Containers should be cleaned. Sterilized packing and grading equipment, particularly brushes and rollers, are used. Chlorinated water @ 100 ppm is commonly used for washing vegetables. This can be done with chlorine gas or with either liquid hypochlorite (pH 6.0–7.0). Containers should not be overfilled, which causes severe damage during stacking. Management of temperature is the most important factor to extend the shelf life of fresh vegetables after harvest. It begins with rapid removal of the field heat by using any of the following cooling methods: hydro-cooling, in-package ice, top icing, evaporative cooling, room cooling, forced air cooling, serpentine forced air cooling, vacuum cooling, and hydro-vacuum cooling. The relative humidity during storage should be maintained at about 85–95% for most fruits and 95–98% for vegetables. Transport vehicles should always be cleaned and sanitized before loading.

Future prospects

Little attention has been paid to produce the commercial formulation of bio agents The biocontrol concept should be popularized at University level. Genetic engineering and other molecular tools should offer a new feasibility for improving selection and characterization of biocontrol Need of mass production, understand their mechanism and also evaluate the environmental factors that favour the rapid growth of bio agents People turning more health conscious biocontrol seems to be best alternative to disease suppression Bioagents bring the disease suppression with no environmental hazards Bioagents needs to be formulated that favour the activity survival of microbe containing in it. Significant advances and commercially available products shall be made available for post-harvest use in future.

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