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FUNGI ASSOCIATED WITH THE SPOILAGE OF SWEET ORANGE (*CITRUS SINENSIS*) FRUITS AND THEIR MANAGEMENT BY BIO-CONTROL AGENTS

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A B S T R A C T

Different Sweet Orange fruits (*Citrus Sinensis*) rot fungi were isolated from rotted fruits.These were *Aspergillus niger*, *Aspergillus flavus, Aspergillus fumigatus, Penicillium digitatum.* During pathogenicity test two fungi *Aspergillus niger* and *Penicillium digitatum* were proved to be most pathogenic. The *Aspergillus niger* fungi during dual culture with *Bacillus substilis, Bacillus substilis srtain2, Pseudomonas fluorescens, Pseudomonas aeruginosa , Bacillus methylotrophicus, Bacillus cereus, Bacillus aryabhattai* and *Streptomyces strain* showed different level of inhibation zones. *Bacillus cereus* was the most effective and caused 71.5% inhibition and *Pseudomonas aeruginosa* was least 53.28% and others *Bacillus substilis, Bacillus substilis srtain2, Pseudomonas fluorescens, Bacillus methylotrophicus , Bacillus aryabhattai* ,and *Streptomyces* caused 68.05%, 64.00%, 70.25%, 59.37%, 57.75% and, 55.25%, respectively. *Penicillium digitatum* fungi during dual culture with *Bacillus substilis, Bacillus substilis srtain2, Pseudomonas fluorescens, Pseudomonas aeruginosa , Bacillus methylotrophicus, Bacillus cereus, Bacillus aryabhattai* and *Streptomyces strain* showed different level of inhibation zones. *Bacillus cereus* was the most effective and caused 69.25% inhabition and *Pseudomonas aeruginosa* was least 51.75% respectively. During experiment on fruits the most effective biocontrol agent *Bacillus cereus* and *Streptomyces strain* were tested on fruits and it was found that *Bacillus cereus* and *Streptomyces strain* were effectively control the rot by *Aspergillus niger* and showed data 49.45%, 43.55%. During experiment on fruits the most effective biocontrol agent *Bacillus cereus* and *Streptomyces strain* were tested on fruits and it was found that *Bacillus cereus* and *Streptomyces strain* were effectively control the rot by *Penicillium digitatum* and showed data (51.62%, 45.67%). Experiment were performed for protective and curative effects. During protective effect the two biocontrol agents *Bacillus methylotrophicus* and *Bacillus cereus* reduced the percentage rottenig by 64.74% and 57.52% respectively in case of *Aspergillus niger* and in case of *Penicillium digitatum* the effect of two Biocontrol agents *Bacillus methylotrophicus* and *Bacillus cereus* caused 59.37% and 54.63% respectively reducuction in decaying. During the Curative effect the two biocontrol agents *Bacillus methylotrophicus* and *Bacillus cereus* reduced the rottenig percentage 62.53% and 55.84% respectively in case of *Aspergillus niger* but in case of *Penicillium digitatum* the effect of the Biocontrol agents *Bacillus methylotrophicus* and *Bacillus cereus* caused 58.29% and 51.99% respectively reducuction in decaying. Use of Biocontrol agents as protective is better than the curative.

Keywords: Strawberry; Runner production; Postharvest quality; Temperate conditions

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INTRODUCTION

Citrus sinensis (L.), family Rutaceae, is one of the major commercial fruit crops that are widely consumed both as fresh fruit or juice due to its high vitamin C content and antioxidant potential (Gorinstein et al., 2001). It is widely cultivated in tropical and subtropical climates, where soil and climatic regions encourage his growth (Shah et al., 2015). Due to its decorative appeal, health advantages and relevance for the fruit juice industries, the production of citrus is growing in Pakistan and around the world. In Pakistan it is the major fruits group growing over 195000 hectares with yearly output of 1986,7000 tonnes, while in Khyber Pakhtunkhwa the area is 43000 hectares, with total output of 335000 tonnes (Pakistan Bureau of Statistics,

2011). Pakistan ranks among the world's top 10 producers for citrus fruit production (Nawaz et al., 2019). Citrus is cultivated on over 170,000 acres, making up around 30 percent of all fruit plantations in Pakistan (Ashraf et al., 2010; Saleem et al., 2008). Pakistan is among the large producer followed by the United State of America (USA), China and Mexico. Spain, USA and South Africa are the largest exporting countries followed by Turkey and Morocco (Citrus commodity notes, 2005).

Sweet orange (*Citrus sinensis* L. Osbeck) (to distinguish it from closely related species like sour orange, *C. aurantium C. reticulata* and mandarin orange), is a small evergreen tree 7.5 m high and in some cases up to 15 m. It originated from southern China where it has been cultivated for many years, but is today grown commercially worldwide in tropical, semi-tropical and some warm temperate regions to become the most widely planted fruit tree in the world (Ehler, 2011; Nicolosi et al., 2000). Orange produces leathery and evergreen leaves of different shapes, ranging from elliptical to oblong to oval, 6.5-15 cm long and 2.5-9.5 cm wide, often bearing narrow wings on the petioles. It bears fragrant white flowers either singly or in whorls of 6, about 5 cm wide, with 5 petals and 20-25 yellow stamens. The small, white or purple scented hermaphroditic flowers produce nectar for pollination by insects. The fruit, which may be globose to oval is 6.5 to 9.5 cm wide, and ripens to orange or yellow. Anatomically, the fruit consists of two distinct regions the pericarp also called the peel, skin or rind, and the endocarp, or pulp and juice sacs. The skin consists of an epidermis of epicuticular wax with numerous small aromatic oil glands that gives it its particular smell. The quantity of wax is dependent on the variety, climatic conditions and growth rate. A plethora of microflora consisting mainly of fungus and bacteria are present on the skin and more copious in damp climates. This justifies the need for appropriate washing of the fruit before eating or proceeding to extract juice and essential oils. The pericarp consists of the outer flavedo, or epicarp largely made of parenchymatous cells and cuticle. Embedded oil glands create terpenoid aromatic compounds such as valencene, limonene, and alpha/beta sinsenal (Goudeau et al., 2008). Beneaththe epidermis is the flavedo, with its characteric yellow, green or orange colour.

Sweet orange is an important fruit crop in international trade next to grapes requiring excellent quality and shelf life attributes. Unfortunately, it is known to be attacked by several pathogens that affect the fruit quality. Citrus fruits are important commercial fruits and widely distributed in the world. It is estimated that the global citrus production in 2017 was up to around 50 million metric tons. Besides good sensorial characteristics, citrus contain high levels of antioxidant compounds, including vitamin C, favanones, and anthocyanin's. However, citrus fruits are exposed to many postharvest diseases during transportation and storage, among which green mold, caused by *Penicillium digitatum*, is one of the most devastating diseases, causing significant economic and resource losses in the world.

Spoilage microorganisms can be introduced into the crop on the seed itself, during crop growth in the field, during harvesting and postharvest handling, or during storage and distribution (Barth et al., 2009). Postharvest losses and decay of citrus fruits can be traced to infections that occur either between flowering and fruit maturity or during harvesting and subsequent handling and storage activities. Pre-harvest infections are mainly caused by fungal pathogens such as *Phytophthora spp, Colletotrichum glocosporiodes* (El Ghaouth et al., 2002). Traditionally, application of synthetic fungicides such as thiabendazole and imazalil was the main method to control green mold, while resulted in pathogen resistance. The biological control of major postharvest pathogens for cereals and fruits was reported by all kinds of microbial antagonists such as *Rhizobacterial isolates Bacillus subtilis*, *Pseudomonas* spp, and so forth (Ali et al., 2014; Hyder et al., 2020; Shahzaman et al., 2016).

The objective of this study was to isolate and identify fungi associated with postharvest deterioration of sweet orange fruits in Rawalpindi and to evaluate the effectiveness of *Rhizobacteria* in the control of citrus green mold caused by *P. digitatum in vitro* via measuring the pathogen colony diameter on agar plates and *in vivo* via calculating the disease incidence on fruits. Moreover the preventive and curative effect of rhizobacteria on the pathogens.

MATERIAL AND METHODS

Citrus fruit samples was randomly obtained from three nearby fruit markets Dhok Kala Khan Sabzi Mandi (Rawalpindi), University Research Farm Koont (Chakwal), and Mandi Mor (Islamabad), and thirty healthy orange fruits were later obtained for the pathogenicity test after the isolation of the fungi. All the samples collected were placed in a sterile polythene bags separately and labeled appropriately and transported to Mycology laboratory, Department of Plant Pathology, PMAS Arid Agriculture University Rawalpindi for the fungal analysis.

Isolation, purification and identification

Cotton wool soaked in 70% alcohol was used to surface sterilise the infected citrus fruit sample..Infected fruit

segments were plated on solidifieded potato dextrose agar plates and were incubated at 28 °C for 7 days. Diverse fungal isolates with different colorations were observed on the incubated plates which indicated different fungal colonies.The fungal clusters those arisen continuously sub-cultured in order to take a pure culture of the fungal isolates.

Slide cultural techniques was used to detect 1 to 4 weeks pure cultures of fungal isolates using social and structural parameters such as colony development pattern, Conidial morphology, and coloring using a light microscope, first with a 10x objective lens and then with a 40x objective lens.

Pathogenicity Test

Using a sterilised 2mm cork borer, tissues were cut from the fruits.One week old fungal culture was inoculated into the holes, and control samples were inoculated with distle water then covered and sealed with petroleum jelly in sterile polythene bagsfollowed by incubation for 14 days at 28 °C.

Dual Culture Technique

Petri dishes (90 mm) containing 20 ml PDA were used in bioassay. Bacterial isolates were grown on Nutrient Broth (NB) at for 24 h to obtain fresh culture. Pathogenic fungal isolates were grown for 5 days at 27 °C on PDA. Bacterial suspensions were individually streaked with a sterile swap on the PDA plates as a circular inner edge of the plate and pathogen fungi was placed in the middle of the petri plates. The plates were wrapped with parafilm followed by incubation at 27 °C until fungal mycelia completely covered the control petri plates. Pathogenic fungi radial growth was measured in mm. Experiment was repeted thrice. The percentage inhibition rate of pathogenic fungi by bioagent bacterial isolates was calculated by using the formula.

Inhibition (%) = (C−T)×100/(C −6)

C: the diameter of the pathogen colony of control group

T: the diameter of pathogen colony after treatments.

Evaluation of biocontrol agents against fruit rot fungi

Citrus fruits were wounded and treated with 20 μL of bacterial suspension and allowed to dry for 2 h. Then, the same volume of 1×10^4 spores/mL conidial suspension of *Aspergillus niger* was inoculated into each wound site with a micropipette. When the surfaces of the fruits were dry, the fruits were put into fresh-keeping bags, and each citrus was put in a fresh-keeping open bag in order to avoid mutual interference. All treated fruits were placed at 20 °C under 90% relative humidity. The lesion diameters were determined by taking the mean of the horizontal and vertical diameters of each lesion, and the disease incidence was calculated by the number of infected wounds. Any fruit wound with visible mold growth was considered to be infected.

Preventative action of *Bacillus cereus* and *Bacillus methylotrophicus* against *Aspergillus niger and P.digitatum*

To study the Preventataive effect of antagonastic rhizobacteria on the fruits, the wounded fruits were treated with 20 μL of 1×10^8 CFU/mL bacterial suspension *Bacillus cereus* and B1 after the wound site had dried for 24 h, each wound was inoculated with 20 μL of 1×10^4 spores/mL conidial suspension of *Aspergillus niger*.The same experiment was treated with *P.digitatum.*

Curative action of *Bacillus cereus* and *Bacillus methylotrophicus* against *Aspergillus niger and P. digitatum*

To study the curative effect of antagonastic rhizobacteria on the fruits the wounded fruits were treated with 20 μL of 1×10^4 spores/ml conidial suspension of *Aspergillus niger*, after the wound site had dried for h, 6h each wound was inoculated with 20 μL of 1×10^8 CFU/mL bacterial suspension *Bacillus cereus* and B1. Then, all treated fruits were packed and placed in a constant temperature and humidity incubator at 20 °C and 90% RH for 8 days afterwards, the lesion diameters and disease incidence were determined as described earlier. The same experiment was treated with *P. digitatum.*

RESULTS AND DISCUSSION

The fruit samples were brought in laboratory associated with citrus many pathogenic fungi were isolated from fruits but mostly *aspergillus niger* and *P. digitatum* were present in majority of fruits. Fungal colonies with white aerial mycelia and concentric rings of black pycnidia various sizes were observed when transferred to fresh PDA medium. Hyphae were septate and conidia were hyaline, one-celled, ellipsoid to fusiform (average 10.1–20.2×3.2–4.3 μm). Morphological character of *Aspergillus niger* isolate on PDA and pathogenicity test results were confirmed. *P. digitatum* was also identified on the basis of morphology. The isolated fungi from the rotten fruits of the *Citrus sinensis* and their frequencies of occurrence are shown in table 1. Collection of infected samples from different markets are shown in table 2.

The study confirmed that *A. Niger, A. flavus, A. fumigatus,* and *P. digitatum* and some yeasts were found in the spoilt sweet orange fruits sold in Rawalpindi, Pakistan. Some of these pathogens have been reportedly isolated from Pawpaw fruits in Nigeria (Baiyewu et al., 2007a; Chukwuka et al., 2010). Out of the fungi isolated, *A. Niger* has the highest frequency of occurrence (36 %) followed

by *A. flavus* (25 %) then *A. fumigatus* (22%) and *p. digitatum* with 35 % frequency of occurrence. This is however in agreement with Bello (2010) whom both isolated about seven different fungal genera from different fruits including sweet orange fruits and when these isolates were aseptically inoculated into healthy susceptible fruits, the characteristic symptoms originally observed were also noticed.

Table 1: Fungi isolated from rotten of *Citrus sinensis* fruits in Rawalpindi.

Fungal Isolates	Frequency (%)
Aspergillus niger	36.0
Aspergillus flavus	25.0
Aspergillus fumigatus	22.0
Penicillium digitatum	35.0

Table 2: Collection of infected samples from different markets.

Pathogens	Areas											
	Dhok Kala Khan Sabzi Mandi (Rawalpindi)				University Research Koont Farm (Chakwal)				Mandi Mor (Islamabad)			
	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
Aspergillus flavus,	+	+	-	-	+	-	+	-	-	+	-	-
Penicillium digitatum	+	+	+	+	+	+	+	+	+	+	+	+
Alternaria alternat	-	-	-	-	+	-	-	-	-	+	-	-
Aspergillus niger	+	+	+	+	+	+	+	+	+	+	+	+
Colletotrichum gloeosporioides	-	-	-	-	-	-	-	-	-	+	+	-
Aspergillus fumigatus	-	-	+	+	-	+	-	+	-	-	+	-
Emericella variecolor	-	-	-	-	-	-	-	-	-	-	-	+
Botrytis cinerea	-	-	-	+	-	-	-	-	-	-	-	+
P. glabrum	-	-	-	+	-	-	-	-	-	-	-	-
Penicillum Italicum	-	+	-	+	-	+	-	-	+	+	-	-

Pathogecity test

The pathogenicity of the isolated fungi from the rotten *Citrus sinensis* fruit after fourteen days of incubation confirmed the fungal pathogens associated with disease (Table 3). The four fungi *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium digitatum* which were mostly isolated from citrus fruit were tested.The results showed that Fungi *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Penicillium digitatum,* were caused 45.0,35.0,30.0,38.0 % fruits damage respectively.

Table 3: Decay rate of fungi isolated from rotten *Citrus sinensis* fruits after 14 days of incubation.

Replications	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus fumigatus*	*Penicillium digitatum*	Water
R1	55.5 mm	38.5 mm	34.3 mm	44.2 mm	0 mm
R2	41.5 mm	32.5 mm	28.7 mm	36.5 mm	0 mm
R3	45.8 mm	36.8 mm	31.6 mm	40.0 mm	0 mm
R4	37.2 mm	32.2 mm	25.4 mm	31.3 mm	0 mm
Mean	45.0 ± 3.92^{a}	35.0 ± 1.57^{bc}	30.0 ± 1.91^{c}	38.0 ± 2.73^{ab}	0.0 ± 0.0^{d}

All the four organisms were successfully taking part in the decay and are thus confirmed as the causal organism of fruit decay (Baiyewu et al., 2007b; Chukwuka et al., 2010).Thus These fungi were also found to be associated with the deterioration of orange fruits, All the four organisms isolated were confirmed to cause spoilage on the sweet orange fruits but

in varying degrees. Of all the isolated fungi, *A. niger* and *P. digitatum* were the most pathogenic with rapid disintegration of the treated fruits in 14 days having a rots diameter of 45mm and 38mm, respectively. And the least pathogenic fungus was *A. fumigatus* having a rots diameter of 30mm.

Some molds may produce mycotoxins (Tournas and Stack, 2001). The fungi isolated in this study have been reported to produce secondary metabolites in plants tissues. These secondary metabolites are potentially harmful to humans and animals (Baiyewu et al., 2007a). A good example is Aflatoxin which has been implicated in cancer of the liver (heplatoma), aflatoxicosis and also acute hepatitis in humans, especially in the developing world (Baiyewu et al., 2007b). Pathogenic fungi, on the other hand, could cause infections or allergies (Mons, 2004).

Dual culture techniques

In vitro inhibition test results of biocontrol agent bacteria isolates were tested against *Aspergillus niger* and *Penicillium digitatum* which is the most prevalent in fruit rot of citrus. The percent inhibition rate in the control was statistically different from all other tested bacteria (Table 4). Percentage inhibition rate values were between 53.32-71.5%. The highest percent inhibition rate was observed in *Bacillus cereus* (71.5%), *Pseudomonas aeruginosa* was least 53.23% and others *Bacillus substilis, B. substilis srtain2, Pseudomonas fluorescens, B. methylotrophicus , Streptomyces strain, B. aryabhattai* and *B4* caused 68%, 64%, 70.25%, 59.37, 57.75%, 55.25%, and 53.28% respectively.

During the dual culture *Bacillus cereus* was most effective bio control against the *Aspergillus niger* similarly Tozlu et al. (2018), tested two isolates of *B. substilis in vitro* and *in vivo* against Sclerotinia sclerotiorum and determined that these isolates were highly effective in preventing the disease.

Table 4: The most effective bio control agent bacteria in the dual culture against *Aspergillus niger, Penicillium digitatum.*

Biocontrol agents	*Aspergillus niger*				Mean
	R1	*R2*	*R3*	*R4*	
Bacillus cereus	*78.0*	*73.0*	*69.5*	*65.5*	71.5 ± 2.65^{a}
Bacillus substilis	*75.5*	*63.5*	*68.7*	*64.5*	68.05 ± 2.73^{ab}
Bacillus substilis srtain2	*69.0*	*61.5*	*62.0*	*63.5*	64.00 ± 1.72^{bc}
Pseudomonas fluorescens	*79.0*	*67.5*	*70.0*	*64.5*	70.25 ± 3.13^{ab}
Bacillus methylotrophicus	*65.5*	*60.5*	*54.5*	*57.0*	59.37 ± 2.38^{cd}
Bacillus aryabhattai	*62.5*	*59.0*	*55.5*	*54.0*	$57.75\pm1.9^{c-e}$
Streptomyces strain	*63.0*	*55.5*	*50.5*	*52.0*	55.25 ± 2.79^{de}
Pseudomonas aeruginosa	*56.0*	*52.5*	*51.0*	*49.5*	52.25 ± 1.4^{e}

Use of bio control agents against fruit rot fungi

The data clarified that minimum colony growth of *Aspergillus niger* and *Penicillium digitatum* (0.20 and 0.30%) was examined while treated against *Bacillus methylotrophicus,Bacillus cereus , Bacillus aryabhattai and Streptomyces strain* at the dosage of 5%, 10% and 15% following (51.62, 49.55 %), (44.25, 44.75), (45.5 , 42.75%), (45.67, 46.55 %) at the dosage of 5%, 10% and 15%, respectively. Maximum colony growth of *Aspergillus niger* (51.62, 49.55) was observed under and *Bacillus methylotrophicus , Bacillus cereus , Bacillus aryabhattai* and *Streptomyces strain* at the dosage of 5%, 10% and 15%. Under control, the *Aspergillus niger* showed (90 mm) colony growth was obbserved (Table 5). On the basis of means, *Bacillus cereus* ranked *1st, Bacillus substilis srtain 2* ranked 2 nd, *Pseudomonas fluorescens* 3rd, *Bacillus substilis* 4th, *Pseudomonas aeruginosa* 5th, *Bacillus methylotrophicus , Bacillus aryabhattai, Streptomyces strain* ranked 6th for controlling colony growth of *Aspergillus niger* and *Penicillum digitatum* under *in-vitro* conditions. Statistical analysis of the obtained data reveals that there was a significant difference in linear colony growth of *Aspergillus nigers* and *P. digitatum* among the Biocontrol agents at different dosages.

During the fruit experiment *Bacillus cereus* and *Bacillus methylotrophicus* which show more antagonist activity against the fruit rot disease .Similarly Al-Hussini et al. (2019) tested that *B.cereus* used against *Pythium aphanidermatum* (damping-off on tomato) and they observed that according to control 27% prevent the disease.

Preventative Action of *Bacillus cereus* and *Bacillus methylotrophicus* against *Aspergillus niger and Penicillum digitatum*

To study the preventative effect of antagonastic rhizobacteria on the fruits, the wounded fruits were

treated with 20 μL of 1 × 10^8 CFU/mL bacterial suspension *Bacillus cereus* and *Bacillus methylotrophicus* after the wound site had dried for 24 h, each wound was inoculated with 20 μL of 1 × 10^4 spores/mL conidial suspension of *Aspergillus niger* and observed 77% control of the disease.The same experiment was treated with *Penicillum digitatum* and was calculated 67% inhibition of fungal growth as compared to control (Table 6).

Protection application of rhizobacterial isolates *Bacillus cereus* and *Bacillus methylotrophicus* were most effective bio control agents against *Aspergillus niger* and *Penicillum digitatum* as results showed.Our results are supported by Gal-Hemed et al. (2011) used biocontrol of *Alternaria alternata* and *Fusarium oxysporum* by *Trichoderma asperelloides* and *Bacillus paralicheniformis* in tomato plants.and also used as protective measurements for control disease.

Table 5: Bio control agents against fruit rot fungi of citrus fruits.

	Penicillium digitatum				Mean
	R1	R2	R3	R4	
T1	56.3	52.00	50.2	49.8	52.075±1.48[a]
T2	53.2	45.6	47.5	48.3	48.65±1.62[ab]
T3	49.5	42.00	45.3	44.00	45.2±1.59[bc]
T4	47.5	37.4	39.00	40.6	41.125±2.22[c]
	Aspergillus niger				
	R1	R2	R3	R4	Mean
T1	55.3	52.4	50.00	51.5	52.3±1.16[a]
T2	50.4	47.00	44.5	46	46.975±1.25[b]
T3	48.00	40.7	39.5	49.2	44.35±2.48[bc]
T4	44.5	39.00	37.00	39.5	40.00±1.59[c]

Table 6: Preventative Action of *Bacillus cereus* and *Bacillus methylotrophicus* against *Aspergillus niger and Penicillum digitatum.*

	Aspergillus niger				
	R1	R2	R3	R4	Mean
Bacillus methylotrophicus	70.20	66.25	60.18	62.35	64.74±2.21[a]
Bacillus cereus	*60.30*	*54.10*	*57.20*	*59.30*	57.72±1.37[ab]
Bacillus aryabhattai	*52.25*	*44.45*	*45.33*	*42.30*	46.08±2.15[c]
Bacillus substilis	*48.30*	*39.00*	*42.50*	*44.85*	43.66±1.95[cd]
Bacillus substilis srtain2	57.38	44.18	51.15	39.40	48.02±3.94[c]
Streptomyces strain	42.22	39.10	33.00	35.50	37.45±2.02[d]
Pseudomonas fluorescens	63.15	52.18	48.90	57.32	55.38±3.11[b]
Pseudomonas aeruginosa	*42.50*	*38.28*	*33.50*	*31.40*	36.42±2.48[d]
	Penicillium digitatum				
	R1	R2	R3	R4	Mean
Bacillus methylotrophicus	68.50	59.29	52.28	57.44	59.37±3.38[a]
Bacillus cereus	58.23	47.52	53.18	59.62	54.63±2.75[ab]
Bacillus aryabhattai	43.28	29.00	33.18	28.20	33.41±3.46[c]
Bacillus substilis	49.30	38.00	41.30	34.50	40.77±3.16[c]
Bacillus substilis srtain2	57.15	51.10	44.70	48.50	50.36±2.62[b]
Streptomyces strain	41.38	39.41	35.00	30.50	36.57±2.42[c]
Pseudomonas fluorescens	53.50	51.16	44.15	48.55	49.34±2.00[b]
Pseudomonas aeruginosa	38.35	31.14	28.70	35.40	33.39±2.15[c]

Curative Action of *Bacillus cereus* and *Bacillus methylotrophicus* against *Aspergillus niger and Penicillum digitatum*

During the curative effect of antagonastic rhizobacteria on the fruits the wounded fruits were treated with 20 μL of 1 × 10^4 spores/ml conidial suspension of *Aspergillus niger*, after the wound site had dried for h, 6h each wound was inoculated with 20 μL of 1 × 10^8 CFU/mL bacterial suspension *Bacillus cereus* and *Bacillus methylotrophicus* the biocontrol agents control 65% fungal growth of *Aspergillus niger* (Table 7). The same experiment was treated with *Penicillum digitatum* and observed 62.80% control. The results of current study are similar with the observation of Gal-Hemed et al. (2011) that mentioned the role of secondary metabolites of several strains from *Trichoderma spp.* to control several plant pathogens, especially in the rhizosphere. *Trichoderma* spp. and *R. solani* interacted with many mechanisms to destroy the hyphae cell wall and the membrane permeability. *T. asperelloides* has been shown to suppress the incidence of *R. solani*, which causes damping-off disease in beans and induces defensin genes in cucumber seedlings against *Pseudomonas syringae pv*. Ramírez-Cariño et al. (2020) used biocontrol of *Alternaria alternata* and *Fusarium oxysporum* by *Trichoderma asperelloides* and *Bacillus paralicheniformis* in tomato plants.

Table 7: Curative Action of *Bacillus cereus* and *Bacillus methylotrophicus* against *Aspergillus niger and Penicillum digitatum.*

	Aspergillus niger				
	R1	R2	R3	R4	Mean
Bacillus methylotrophicus	67.25	64.35	60.16	58.38	62.53±2.00^{a}
Bacillus cereus	62.50	56.19	53.30	51.38	55.84±2.43^{ab}
Bacillus aryabhattai	51.22	42.55	40.32	39.38	43.36±2.70^{cd}
Bacillus substilis	46.33	44.00	42.40	37.75	42.62±1.81^{cd}
Bacillus substilis srtain2	54.37	41.15	49.21	38.50	45.80±3.65^{c}
Streptomyces strain	41.32	38.15	34.00	32.60	36.51±1.99^{d}
Pseudomonas fluorescens	64.18	50.22	47.70	55.30	54.35±3.64^{b}
Pseudomonas aeruginosa	40.52	37.23	34.90	30.50	35.78±2.10^{d}
	Penicillium digitatum				
	R1	R2	R3	R4	Mean
Bacillus methylotrophicus	65.40	61.20	51.25	55.34	58.29±3.13^{a}
Bacillus cereus	55.11	48.92	50.28	53.65	51.99±1.45^{ab}
Bacillus aryabhattai	40.27	31.00	34.15	35.29	35.17±1.92^{de}
Bacillus substilis	47.35	39.52	43.37	37.30	41.88±2.21^{cd}
Bacillus substilis srtain2	55.25	49.18	46.72	39.20	47.58±3.32^{bc}
Streptomyces strain	38.18	33.51	31.12	29.40	33.05±1.90^{e}
Pseudomonas fluorescens	52.70	50.14	42.18	46.58	47.9±2.28^{bc}
Pseudomonas aeruginosa	37.55	33.21	28.50	31.30	32.64±1.901^{e}

CONCLUSION

It was found that *A. fumigatus, A. Niger, A. flavus* and *P. digitatum* are detected in spoilt sweet oranges. Therefore, sweet orange fruits should be properly refrigerated below 4 ℃ and should be discarded if any changes noticed in the colour or taste of the fruit as will be hazardous to human health.

AUTHORS' CONTRIBUTIONS

All authors contribute equally for collection, conducting experiment, data collection and analyses and in write-up of this manuscript.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

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