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First Record of *Mucor piriformis* as a Cause of Strawberry Fruit Rot in Iraq and the Effectiveness of Some Safe Strategies in Controlling It and Preventing the Production of Mycotoxins in Stored Fruit: Implications for Food Safety and Postharvest Quality

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ABSTRACT

The purpose of this research was to make a biological study of strawberry fruit rot in Iraq, identify these diseases causing fungi, isolate and characterise the fungal isolates of the Mucor piriformis, and investigate the potential of the fungus for producing mycotoxins which may have a role in food safety and post harvest quality. The study also evaluated the impact of Lactobacillus delbrueckii subsp. bulgaricus, S. cerevisiae, coriander extract, food-grade gelatin, beeswax and the food-grade chemical compound calcium propionate on protecting strawberry fruits from attack by M. piriformis during storage and the prevention of mycotoxin production, particularly in this case. All samples taken from strawberries fruits (from various parts of Iraq) were contaminated and the results of isolation and identification revealed 100% contamination. Twenty-eight-five number of fungal isolates of different fungal genera were isolated and identified in the results. M. piriformis was the most common and abundant fungus. A few isolates were characterized as very virulent strains that cause the disease. The morphological identification of M. piriformis (Kr1Ms) was supported by molecular analyses. The mRNA and genomic sequences corresponding generated deposited in NCBI database with accession number PZ281969.1. More important, the results from the GC-MS analysis indicated that none of the M. piriformis isolates had detectable levels of mycotoxins, indicating that the main threat to food safety from this pathogen is the quality impairment that it causes rather than the contamination of food with mycotoxins. However, for the storage experiment, all treatments which contained Bees wax, gelatin (Je), coriander extract (Cs), calcium propionate (Cp), bacteria/yeast + gelatin (Sc+Ld+Je) and integrating all the treatments (Sc+Ld+Je+Cs+Cp+Path) with 0.00% infection rate in the first week, while the control treatments recorded 100%. At the end of the second week, the integrated treatment (Sc+Ld+Je+Cs+Cp+Path) was 0.00% infected compared to the control treatments which registered a 100% infection rate. There was no difference with these factor for them to be efficient and effective in reducing infection during the third week, the highest infection percentage was 93.75% for pathogen only treatment while the least of the infection was 43.75% for the integrated treatment. The results indicate that combined biological and natural control can successfully extend the shelf life and quality of the strawberries during storage for up to 14 days, which has immense implications for reducing the food waste and ensure the food safety in the fresh produce supply chain..

1- INTRODUCTION

Strawberries (*Fragaria* × *ananassa Duch*.) are among the most delicious and aromatic fruits in the world and have a special status among all the kinds of berries for their attractive red colour, high nutritional value, content of the important active compounds, minerals and vitamins [1]. Fungus pathogens are the main cause of late blight and cause postharvest losses to strawberries, which severely affect the food quality, safety and economy [2, 3]. Quality and safety of fresh strawberries during storage is a fundamental aspect from food science point of view with the aim of minimizing food waste, consumer safety and fresh food science. Maintenance of quality and safety of fresh strawberries during storage is a fundamental aspect of food science to reduce food waste, ensure consumer safety and support the fresh produce science industry [4].

Fruit rots of *Botrytis* spp., *Aspergillus* spp., *Mucor* spp., *Penicillium* spp., *Phytophthora* sp., *Alternaria* sp., and *Rhizopus* sp. are the most important diseases of strawberries after harvest. [5, 6]. Many of these fungi can cause spoilage and/or the production of mycotoxins (toxic secondary metabolites) that have significant health implications for humans and a public health problem since they present in food supply [7, 8]. These diseases result in losses of crop yield, reduced quality and marketability of crops, and potential human disease outbreaks [9].

The widespread use of chemical pesticides for controlling these pathogens is concerning both food safety, human health and environmental sustainability issues. Hence, in recent years, researchers have dedicated their work to understanding the importance of beneficial microbial communities which could be used to suppress pathogens, induce systemic resistance, and hence improve the quality or fruit sugar (sweetness) and hence the sustainable and environmentally friendly management of strawberry crops. These include using the yeast Saccharomyces cerevisiae and bacteria of the genus *Lactobacillus* spp. to control losses caused by spoilage during post harvest in stored fruits [11, 12]. By minimizing the amount of fungi on fruits and the subsequent production of mycotoxins, as well as ensuring fruit quality during storage, fruit quality can also be improved and extended [13].

Much work has also gone into determining alternative, safe, non-toxic and environmentally friendly methods of controlling pathogens. The fungicidal activity of plants has been assessed using plant extracts, with the view that they can be used for controlling fungal diseases [14]. A wide variety of natural antimicrobial agents have been documented and reported in various herbs and plant species including coriander, moringa, sage, senna, clove and others [15, 16]. Medicinal plants might have different concentrations of such biologically active substances that could have a positive antibacterial and antifungal effect, which would be useful in food preservation [17, 18].

For agricultural products, some food additives have also been studied to assess their potential for pathogen control. World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) have officially approved the use of calcium propionate in food items [19]. It has inhibitory effect on the growth and multiplication of a wide range of fungi and bacterial species and so extends the shelf life of food commodities [20]. *Penicillium* spp., *Scopulariopsis* spp., *Mucor* spp., *Fusarium* spp. and *Aspergillus* spp. were significantly inhibited (more than 50%) at 0.20% concentration [21].

Although strawberries are important commercial perishable crop in Iraq and the research is known and need to find safe

effective post harvest preservation methods, there is few information on the occurrence of Mucor piriformis in strawberries and the effectiveness of integrated application of natural and biological control methods for the preservation of fruit quality and safety during storage. There is a research need for determining the levels of this pathogen, the potential to contaminate foods with mycotoxins and the effectiveness of food grade control measures to improve the shelf-life and safety of foods.

The objectives of this study were to: (1) survey and isolate fungal pathogens causing fruit rot in strawberries in Iraq in order to obtain the mycotoxigenic potential of *M. piriformis*; (2) screen the in vitro antifungal activity of *L. delbrueckii* subsp. bulgaricus, *S. cerevisiae*, plant extracts and calcium propionate; (3) protect stored strawberries from fungal attack using integrated treatment and preserve the food quality during refrigeration storage.

We hypothesized that: (1) *M. piriformis* can be considered as one of the most major pathogens of strawberries in Iraq; (2) the isolates can be distinguished according to their ability to produce mycotoxins, and (3) integrated biological and natural control strategies would be able to suppress the fungal contamination and prolong the shelf life of stored strawberries.

2- MATERIALS AND METHODS

2.1 Collection of Samples Infected with Strawberry Fruit Rot Disease

Fruit samples of strawberry were taken from six different sites in Iraq. Local strawberry fruits were randomly picked directly from the strawberry farms in the four governorates (Karbala, Najaf, Sulaymaniyah, and Kirkuk) while the imported fruits were collected from local fruit markets to obtain the survey results that covers all strawberry fruit rot cases. Two subsamples were taken at each site at the rate of 2-3 kg per sample. After collecting the samples, they were placed in bags with plant samples code, sampling place and sampling date, then transported to the Laboratory of Plant Diseases and Mycotoxins, Department of Plant Protection (University of Karbala), College of Agriculture.

2.2. Isolation and Taxonomic Characterisation of Fungal Pathogens

The fungi related to strawberry fruit rot were isolated from the fruits by the standard mycological methods from the symptomatic fruits [22]. Infected material was well washed and surface-sterelised for 1 minute in water containing 2% sodium hypochlorite. The aseptically transferred pieces of tissue were placed onto potato dextrose agar (PDA) medium and five pieces of tissue were used per dish, that is, 100 rose strawberry pieces per sample. The dishes were incubated at 25 ± 2 °C. Growing colonies of fungi were inspected under the microscope for identification after four days using taxonomic keys [23, 24, 25, 26].

2.3 Pathogenicity Test

Six fungal isolates were isolated in this study and a pathogenicity test was carried out on these isolates. An experiment was performed for refrigerated storage, healthy strawberry fruits were used in plastic containers. Eight healthy strawberry fruits were kept in each experimental unit. Surface sterilisation was followed by artificial inoculation of fruits with fungal isolates which were sprayed on a suspension of 1×10^6 spores mL^{-1} (3 mL per

isolate per replicate, three replicates). Three replicates were used as a control treatment and sprayed with sterile distilled water. The results of the tests were evaluated after 14 days incubation at 22 °C.

2.4. In the second step, the ability to produce mycotoxins will be detected.

Pathogenic fungal isolates grown in strawberry fruits were ground and dried, after which, the extraction of mycotoxins was achieved by the method of Hackbart et al. [27]. The detection of mycotoxins was performed by GC-MS technology as described by Zou et al. [28] both qualitatively and quantitatively.

2.5 Molecular Identification

The fungal isolate M. piriformis (Kr1Ms) was identified by sequence analysis of a tip with known sequences, the Internal Transcribed Spacer (ITS), in the fungal genetic region [29]. Isolation of the bacteria Lactobacillus from the genetic 16S region of the section of the dairy yogurt confirmed the bacterial isolate. Nucleotide sequences obtained from Macrogen Company (South Korea) was used for comparing with the data stored in NCBI genbank through BLAST program. A Neighbor-Joining [32] based phylogenetic tree was built from the data using MEGA X version 11 software.

2.6. This involves several steps of processing that that the student should not miss.

Extracts were prepared from eight plants; sage (*Salvia officinalis*), coriander (*Coriandrum sativum*), clove (*Syzygium aromaticum*), moringa (*Moringa oleifera*), senna (*Senna alexandrina*), hibiscus (*Hibiscus sabdariffa*), betony (*Stachys officinalis*) and neem (*Azadirachta indica*) [33]. 35 g was weighed out and transferred to a glass flask (500 mL) that contained 100 mL distilled water. The mixture was shaken for 24 hours at 35 °C, filtered through Whatman No.1 filter paper and then centrifuged at 15,000 rpm for 10 minutes. The extract was then filtered through microfilters (0.22 µm) as a mechanical process to sterilize it.

2.7 In Vitro Antifungal Activity Testing

L. delbrueckii subsp. *bulgaricus*, *S. cerevisiae*, plant extracts and calcium propionate were tested for their inhibitory effects using the poison food technique against M. piriformis [34]. Biological agents were serially diluted and 1 mL of all the dilutions was poured onto PDA plates. An inoculum consisting of 0.5 cm disc from a 7-day-old culture of fungus was placed in the centre of each dish. In the case of the plant extracts, 2mL/100 mls of PDA medium is used. 1, 2 and 3 g L^{-1} of calcium propionate were tested. The percentage of inhibition was calculated as below:

The percentage inhibition is calculated as follows:
% Inhibition = [(Mean radial growth in control – Mean radial growth in treatment) / Mean radial growth in control] × 100

2.8 Storage Experiment

Following laboratory testing, calcium propionate was selected along with coriander extract, sixth number dilution of *S. cerevisiae, L. delbrueckii* subsp. *bulgaricus*, food-grade gelatin and beeswax. The effect of all factors was done in the presence and absence of the pathogenic fungus as per Table 1. There were three replicates per experimental unit with the total number of strawberry fruits per unit being eight for each experimental unit.

2. Each dip was with strawberry fruits separate from the other 24 hours prior to inoculation with the pathogenic fungal suspension. Fruit subsequently was challenged artificially with 3 mL of fungal suspension on the subsequent day, per replicate. Percentages of infection and disease severity were determined and compared with control treatments after 7, 14 and 21 days. Fruits of selected

treatments were dried, ground and used for extraction and determination of the mycotoxins.

Table 1. Treatments Applied to Strawberry Fruits Contaminated with Pathogenic Fungi

No.	Treatment Code	Description
1	Control	Untreated fruits
2	Path only	Pathogenic fungus only
3	Je + Path	Gelatin + pathogen
4	Sc + Path	*S. cerevisiae* + pathogen
5	Ld + Path	*L. delbrueckii* + pathogen
6	Cs + Path	Coriander extract + pathogen
7	Cp + Path	Calcium propionate + pathogen
8	Bw + Path	Beeswax + pathogen
9	Sc+Ld+Je+Path	Yeast + bacteria + gelatin + pathogen
10	Cs+Cp+Path	Coriander extract + calcium propionate + pathogen
11	Sc+Ld+Je+Cs+Path	Yeast + bacteria + gelatin + plant extract + pathogen
12	Sc+Ld+Je+Cs+Cp+Path	All factors + pathogen

2.9 Statistical Analysis

The analysis of variance (ANOVA) with completely randomized design (CRD) [35] was used to analyze the data. The least significant difference test at the 5% probability level [36] was used to perform mean comparisons.

3- RESULTS AND DISCUSSION

The fungi pathogens are isolated and identified in accordance with the size and types of the orchids and their origin.

The contamination from infected strawberry fruits confirmed by the isolation and identification results indicated that the fruits of the infected strawberry variety were contaminated in all of the samples collected (Table 2). Samples were collected from 6 samples representing 6 samples collected from four governorates (Karbala, Najaf, Kirkuk and Sulaymaniyah) and showed contamination rate of 100%. 285 fungal isolates of several fungal genera were isolated and identified. The most common fungus was *Aspergillus* spp. (17.54%) and Mucor spp was next most common fungus. (21.75%), whereas *Botrytis* sp. ranked third (11.23%).

The results were similar to those of El-Shahir et al. [37] who suggested that *A. niger, B. cinerea* and *Mucor* spp. are the most significant causal agents and trigger different diseases affecting strawberry fruits throughout the world. Many pathogenic fungi attack strawberry fruits throughout storage, causing a myriad of diseases and stamford et al. [38] suggested that. Dwivedi et al. [39] found that, *Botrytis cinerea*, *A. niger*, *Colletotrichum* spp., *Rhizopus* spp., *Fusarium* spp., and *Phytophthora* spp. are the most significant pathogens attacking strawberry fruits.

The fungi found on strawberries are a serious issue to consider when assessing food quality because they are not only a cause of spoilage, but can also produce mycotoxins, reducing the quality of food and thereby affecting food intake safety [40]. The high contamination rate (100%) indicates that strawberry is a highly susceptible fruit to fungal attacks during post harvest handling and storage.

Table 2. Fungi isolated from strawberry fruits infected with fruit rot disease

N	Isolated Fungi	Karbala	Kirkuk	Sulaymaniyah	Najaf	Total		
		Husseiniya	Central	Laylan	Penjwen	Nida 1	Nida 2	
	Sample Code	K1	K2	Kr1	S1	N1	N2	
1	*Mucor* spp.	13	11	7	18	6	7	62
2	*A. niger*	12	8	6	4	13	7	50
3	*A. fumigatus*	6	6	2	0	1	4	19
4	*A. flavus*	2	7	0	0	2	4	15
5	*A. ochraceus*	5	0	0	0	2	0	7
6	*Aspergillus* spp.	2	0	5	8	4	1	20
7	*Botrytis cinerea*	7	3	4	5	7	6	32
8	*Penicillium citrinum*	1	0	3	0	2	3	9
9	*P. expansum*	0	0	4	2	2	0	8
10	*P. italicum*	0	2	1	0	0	2	5
11	*Penicillium* spp.	7	5	6	1	1	8	28
12	*Rhizopus* sp.	3	3	1	1	3	0	11
13	*Alternaria alternata*	1	4	1	2	0	1	9
14	*Phytophthora* sp.	0	0	0	1	0	1	2
15	*Trichoderma* sp.	0	0	1	2	0	1	4
16	*Cladosporium* sp	0	2	1	0	0	1	4

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Tota l	59	51	42	44	43	46	28 5

Table 3. Percentage occurrence and frequency of fungi isolated from strawberry fruits

ID	Isolated Fungi	Total Isolates	Occurrence (%)	Frequency (%)
1	*Mucor* spp.	62	100	21.75
2	*A. niger*	50	100	17.54
3	*A. fumigatus*	19	83.33	6.66
4	*A. flavus*	15	66.66	5.26
5	*A. ochraceus*	7	33.33	2.45
6	*Aspergillus* spp.	20	83.33	7.01
7	*Botrytis cinerea*	32	100	11.23
8	*Penicillium citrinum*	9	66.66	3.15
9	*P. expansum*	8	50	2.80
10	*P. italicum*	5	50	1.75
11	*Penicillium* spp.	28	100	9.82
12	*Rhizopus* sp.	11	83.33	3.85
13	*Alternaria alternata*	9	83.33	3.15
14	*Phytophthora* sp.	2	33.33	0.70
15	*Trichoderma* sp.	4	50	1.40
16	*Cladosporium* sp.	4	50	1.40

3.2 Pathogenicity of the Studied Fungal Isolates

It revealed that all the tested fungi were responsible for infection at 100% infection percentage (Table 4). There was significant variation in the effect on disease severity of fungal isolates tested. *Mucor* sp. (100%) indicated high virulence with three fungal isolates, whereas three isolates exhibited moderate virulence and three isolates resulted in low virulence with mean disease severity values of 50%, 75% and 75% respectively. (Kr1Ms), and 52% and 42% for isolates S1.Ms and N2.Ms, respectively. The three fungal isolates were chosen for testing production of mycotoxins. The most virulent isolate (Kr1Ms) was chosen for further experiments.

This also confirms with many studies showing that *M. piriformis* is one of the

most prevalent fungi found with strawberry fruit deterioration [41], especially in post harvest. It characteristically invades the fruit and creates rot and decreases market quality. Its temperature tolerance can be a significant problem for the raw materials of agriculture and for the crops after harvesting even under refrigerating conditions [42].

Table 4. Infection percentage and disease severity in the pathogenicity test of selected fungal isolates

No.	Fungal Isolate	Origin	Isolate Code	Infection (%)	Disease Severity (%)
1	M. piriformis1	K1	K1.Ms	100	15
2	M. piriformis2	K2	K2.Ms	100	20
3	M. piriformis3	Kr1	Kr1.Ms	100	100
4	M. piriformis4	S1	S1.Ms	100	52
5	M. piriformis5	N1	N1.Ms	100	20
6	M. piriformis6	N2	N2.Ms	100	42
LSD 0.05					11.76

3.3 Assessment of Mycotoxin Production

The three isolates of *Mucor piriformis* detected in this study have not been capable of synthesis of mycotoxins as detected by Gas chromatography Mass Spectrometry (GC-MS) analysis: mycoricin, oxalic acid, or other toxins. The discovery is important for food safety because it has shown that the main food safety problem encountered with this pathogen in Iraqi strawberries is related to spoilage and affects food quality more than polyps do. The presence of the pathogen, however, will still affect the market value, shelf life and quality of the fruits, thus rendering control measures important for preserving food quality. The isolate of *Mucor* spp was used in further research experiments after the most virulent one (Kr1Ms).

3.4 Molecular Identification

Based on nucleotide sequences of the *M. piriformis* isolate (Kr1Ms), several genetic similarities were determined with all the 20 fungal *M. piriformis* reference isolates, ranging from 98.59% to 89.78% similarity (Table 5). The phylogenetic tree (figure 1) indicates that this isolate is very similar to the Hungarian isolate (AJ278359.1) and Australian isolate (KP714575). It is also found to be secondarily related to the Indian isolate (PZ006298.1). Genetic divergence results in different clades in it when compared with other isolates like French (JN115816.1), German (EU484276.1), and Dutch (MH854921.1).

The region of the genome designated ITS for fungal species identification in order to investigate the phylogenetic relationships between species is the highly conserved intergenic space between the ribosomal subunits. The molecular identification supports the first recording of the pathogen *M. piriformis* leading strawberry fruit rot in Iraq.

Table 5. Comparison of similarity percentages of ITS gene region sequences of *M. piriformis* isolate Y.n.198.Dheba with global isolates

N	Fungal Name	Isolate Name	Origin	Accession No.	Similarity	Date

1	*M. piriformis*	Y.n.198.Dheba	Iraq	PZ281969.1	100%	2026
2	*M. piriformis*	CBS175.27	Netherlands	MH854921.1	98.59%	2026
3	*M. piriformis*	CBS169.25	Germany	EU484276.1	98.18%	2009
4	*M. piriformis*	NRRL26211	Hungary	AJ278359.1	95.65%	2000
5	*M. piriformis*	GW-OTU47	Austria	KP714575.1	89.78%	2016
6	*M. piriformis*	Com1732	India	PZ006298.1	90.32%	2026

3.5 In Vitro Antifungal Efficacy of Plant Extracts

It was found that coriander extract and clove extract are considered the most effective than other extracts since they recorded the highest level of fungal inhibition of about 85.66% and 77.34%, respectively (Table 6). The following extracts were the result of the other ones. Hence, Coriander extract was chosen as the subject for further studies.

These outcomes were consistent with those obtained from earlier studies which quantified the effectiveness of extracts [44]. The coriander extracts demonstrated antifungal activity against some pathogenic fungi, which resulted in inhibition of fungal growth and decrease in disease severity [45]. This was attributed to the coriander's phenolic and terpenoid contents, the bioactive components against various phytopathogens [46].

Table 6. Effect of plant extracts against the in vitro growth of *M. piriformis*

N	Treatment	Inhibition (%)
1	Moringa extract + *M. piriformis*	36.78
2	**Coriander extract + *M. piriformis***	**85.66**
3	Sage extract + *M. piriformis*	58.65
4	Senna extract + *M. piriformis*	40.43
5	Hibiscus extract + *M. piriformis*	56.66
6	Clove extract + *M. piriformis*	77.34
7	Balbanum extract + *M. piriformis*	40.43
8	Neem extract + *M. piriformis*	46.66
9	*M. piriformis* only (control)	0.00
LSD 0.05		**17.33**

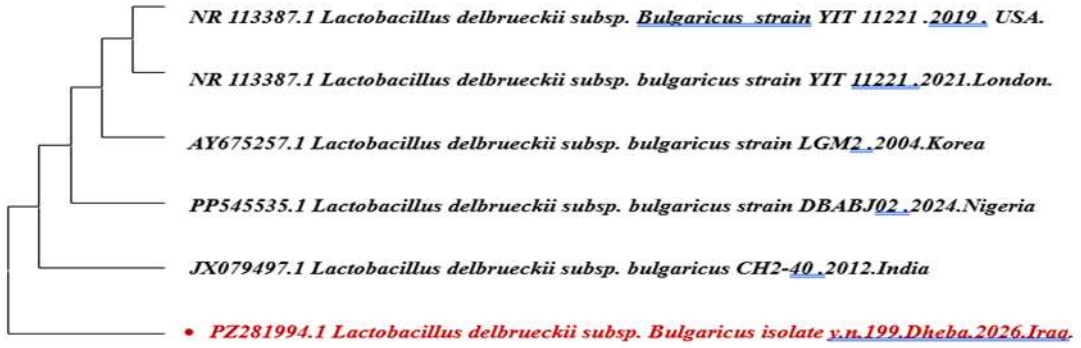

Figure (2) The genetic tree of Lactobacillus delbrueckii subsp. Bulgaricus isolate y.n.199.Dheba

3.6 In Vitro Efficacy of Probiotic Biological Agents

The sixth dilution of yeast suspension showed excellent inhibition of proliferation of *M. piriformis* with an inhibition rate of 87.34% (Table 7). Thus, the 6th dilution was chosen for further experiments.

The inhibition rate of the sixth dilution of *L. delbrueckii* subsp. *bulgaricus* was 83.33%. So it was decided that it would be the sixth dilution to be used in future experiments.

The findings are consistent with that of Jiao et al. [47] who reported that apart from post harvested rot pathogen, yeasts play an important role in biological control. Beneficial yeasts, such as Saccharomyces cerevisiae, have been suggested as biological control agents for postharvest disease pathogens due to their ability to suppress and inhibit these pathogens using various mechanisms such as nutrient and space limitation, secretion of volatiles and cell wall degrading enzymes.

On the other hand, lactic acid bacteria exhibited high inhibition percentage of plant disease-causing fungi, especially *M. piriformis*, and their high inhibitory ability was shown by Kowalska et al. [49] on lactic acid bacteria.

Table 7. Evaluation of probiotic biological agents against in vitro growth of *M. piriformis*

Treatment	Concentration	Inhibition (%)
S. cerevisiae + *M. piriformis*	0.00	0.00
	1×10^{-6}	87.34
	1×10^{-8}	83.33
	1×10^{-10}	77.77
L. delbrueckii + *M. piriformis*	0.00	0.00
	1×10^{-6}	83.33
	1×10^{-7}	77.77
	1×10^{-8}	72.33
LSD 0.05		8.02

3.7 In Vitro Efficacy of Calcium Propionate

The results showed that calcium propionate was inhibitory but the inhibitory action was differential and related proportionately with the increase in concentrations of pathogenic fungal isolates. It was found that the optimal concentration giving the highest inhibition was 0.3% (Table 8) while the lowest was 0.00% (control).

Ca propionate has been proven to be safe to use as a prevention and control of crop post-harvest fungi infection in previous studies [19]. Kaur et al. [20] said that calcium propionate was found to be effective in the suppression of fungal pathogens including fungicide resistant fungi.

Table 8. Evaluation of calcium propionate against in vitro growth of *M. piriformis*

Treatment	Concentration	Inhibition (%)
Calcium propionate + *M. piriformis*	0.0%	0.00
	0.1%	83.33
	0.2%	94.44
	0.3%	100.00
LSD 0.05		9.67

3.8 Effect of Integrated Treatments on Strawberry Fruit Rot During Storage

The results in the infection percentage after 7, 14 and 21 days of infection (Table 9) indicated that the general mean percentage of infection were 12.50%, 30.68%, and 100% respectively. This showed that, in general, this treatment prevented the growth of this pathogen in the first and second weeks, but was unable to maintain fruit health in the third week.

In the first week, some treatments were entirely effective against infection (0.00%) as compared to control treatments (100%): (Cs+Ld+Je+Cp+Cp+Path): Treatment with bacteria and yeast, gelatin, calcium propionate and beeswax were most effective, gelatin alone and calcium propionate alone and beeswax alone.

In the second week, control (100%) had no infection controlled whereas integrated treatment (Sc+Ld+Je+ Cs+Path) completely controlled the infection (0.00). After 3 weeks, however, all treatments yielded 100% infection rate, with none of these conditions being effective in reducing infection.

The general mean value in the results of the disease severity after 7, 14 and 21 days (Table 10) were 3.98%, 9.66% and 75.85%, respectively. The pathogen-only treatment recorded the highest disease severity in the 3rd week (93.75%) while the lowest disease severity occurred in the integrated treatment (43.75%).

Such findings are in accord with those of Godana et al. [50] who suggested that combined use of biological and natural control agents has been found to be effective in protecting postharvest crops against postharvest rots but will not be as effective for prolonged storage periods. The integrated technology had superior effectiveness during fortnight one and two, because of a synergistic interaction between the biological and natural technologies they are based on, which helped to control *M. piriformis* growth and to decrease the colonization of fruits [51]. In the third week, however, the efficiency of all treatments dropped possibly because any biological and natural substances lost their efficiency as more pathogens were active.

In terms of food quality, the integrated treatment resulted in strawberries with a slightly longer shelf life of about 14 days than any of the other options, potentially increasing economic value and improving food value chain efficiency by decreasing food wastage [52].

Table 9. Effectiveness of treatments in reducing strawberry fruit rot infection percentage during storage

N	Treatment	After 7 Days		After 14 Days		After 21 Days	
		Without Path	*M. piriformis*	Without Path	*M. piriformis*	Without Path	*M. piriformis*
1	Path only	0.00	100	25	100	50.0	100
2	Je + Path	0.00	0.00	0.0	37.5	37.5	100
3	Sc+Je+Path	0.00	0.00	12.5	37.5	25	100
4	Ld+Je+Path	0.00	12.50	12.5	37.5	12.5	100
5	Cs+Path	0.00	0.00	12.5	37.5	25	100
6	Cp+Path	0.00	0.00	0	25	12.5	100
7	Bw+Path	0.00	0.00	0	25	12.5	100
8	Sc+Ld+Je+Path	0.00	0.00	12.5	0.00	25	100
9	Cs+Cp+Path	0.00	12.5	0	37.5	0	100
10	Sc+Ld+Je+Cs+Path	0.00	12.5	0	0.00	12.5	100
11	Sc+Ld+Je+Cs+Cp+Path	0.00	0.00	0.0	0.00	0	100
Treatment Rate		0.00	12.50	6.82	30.68	19.32	100.00
LSD 0.05			0.23	2.54	11.89	9.23	

Table 10. Effectiveness of treatments in reducing disease severity of strawberry fruit rot during storage

N	Treatment	After 7 Days		After 14 Days		After 21 Days	
		Without Path	*M. piriformis*	Without Path	*M. piriformis*	Without Path	*M. piriformis*
1	Path only	0.00	25.0	9.37	37.50	12.5	93.75
2	Je + Path	0.00	0.00	0.00	9.37	9.37	93.75
3	Sc+Je+Path	0.00	0.00	3.12	9.37	6.25	100
4	Ld+Je+Path	0.00	9.37	3.12	18.75	6.25	81.25
5	Cs+Path	0.00	0.00	3.12	9.37	3.12	62.50
6	Cp+Path	0.00	0.00	0.00	6.25	3.12	75.00
7	Bw+Path	0.00	0.00	0.00	6.26	3.12	84.37
8	Sc+Ld+Je+Path	0.00	0.00	3.12	0.00	6.25	87.50
9	Cs+Cp+Path	0.00	0.00	0.00	9.37	0	56.25
10	Sc+Ld+Je+Cs+Path	0.00	9.37	0.00	0.00	3.12	56.25
11	Sc+Ld+Je+Cs+Cp+Path	0.00	0.00	0.00	0.00	0	43.75
Treatment Rate		0.00	3.98	1.99	9.66	4.83	75.85
LSD 0.05			1.25	1.56	8.89	3.23	7.78

3.9 Food Quality and Safety Implications

The results from this study has wide applications in the fields of food quality, food safety and for the fresh produce industry:

3.9.1 Mycotoxin Safety

This has great positive news for food safety: that M. piriformis isolates failed to produce detectable levels of mycotoxins. Food safety issues in fresh produce include mycotoxins like *afalatoxins, ochratoxins* and *patulin* [53]. The pathogen produces no mycotoxins so the greatest concern for strawberry safety is degradation of quality associated with spoilage, but it's advisable to continue monitoring [54].

3.9.2 Shelf Life Extension

Internal treatment has lengthened the shelf-life of strawberries by about 14 days when stored under refrigerated storage conditions. It is a great achievement that can: (1) lower the food waste and economic losses; (2) make strawberries marketable for a longer period of time; (3) improve the efficiency of the strawberry supply chain; and (4) increase consumer satisfaction from product quality [55].

3.9.3 Clean Label and Consumer Acceptance

The application of food-grade, natural and biological control agents is in line with the need for clean label products for consumers demand [56]. The different treatments tested in this study are: *S. cerevisiae* (a food grade yeast for baking and fermenting products); *L. delbrueckii* subsp. *bulgaricus* (a probiotic bacterian used in yogurt production); coriander extract (a food ingredient); gelatin (a food additive); beeswax (a natural coating); and calcium propionate (a food preservative). Most of these ingredients are generally recognized as safe (GRAS) to be used in food, and easily accepted for minimally processed foods by consumers [57].

In the end, a new method for reducing chemical preservants was developed Use of biological and natural control agents in this study will contribute to the reduction of synthetic chemical preservatives in fresh produce [58]. This matches the trend for regulations and consumer preference on the diminishing amount of synthetic pesticides and preservatives used in foods [59].

4-CONCLUSIONS

M. piriformis was recently reported to be widely present in strawberries and in this study it was confirmed as the cause of strawberry fruit rot for the first time in Iraq. The isolate selected demonstrated good disease inducing potential and no production of mycotoxins indicating that the major health and safety issue regarding food is the deterioration of quality caused by spoilage.

The following conclusions can be made:

1. Coriander extracts 85.66%, S. cerevisiae 87.34%, *L. delbrueckii subsp. bulgaricus* 83.33% and at 0.3% calcium propionate 100% inhibition were the best treatments in in vitro experiments.
2. The integrated treatment (Sc+Ld+Je+Cs+Cp+Path) showed highest efficacy under storage condition; it was completely disease free up to 2 WAP and 43.75% disease was reduced after 21 days (93.75 % reduction in outbreak in the control).
3. The integrated treatment increased the shelf life of strawberries by about 14 days, which provides great quality of food, minimises food waste and adds economic value.
4. The use of food-grade, natural and biological control agents is in keeping with the clean label preference by the consumer, and assists in reducing the use of synthetic chemical preservatives.
5. Lack of mycotoxin production by *M. piriformis* isolates is a good trend in relation to food safety, but is

recommended to monitor it continuously.

6. The use of beneficial microorganisms together with natural and food-safe materials appear to be a safe and ecological solution for strawberry fruit rot control for short storage, which have great potential for improving food quality, safety and sustainability.

5. RECOMMENDATIONS

From the result of this study the following recommendations are made;

1. For commercial use, the integrated treatment (Sc+Ld+Je+Cs+Cp+Path) is recommended to help prolong the shelf life of strawberry during refrigerated storage.
2. Food grade calcium propionate (0.3%) is recommended as a safe preservative for strawberries, according to WHO/FAO regulations.
3. Addition: Further studies are needed to explore coriander extract as antifungal natural preservative to extend shelf life in fresh produce.
4. Future research should include; (a) testing sensory properties of treated strawberries, (b) consumer acceptability, (c) economic analysis, and (d) mechanism of action.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Data Availability

All data supporting the findings of this study are available within the manuscript.

Ethical Approval

This study did not involve human participants or animals. All experimental procedures were conducted in accordance with institutional and laboratory biosafety guidelines.

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