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ORIGINAL ARTICLE

Anti-sporulation effectiveness of leaf extracts of three *Prosopis* species on spoiling fungi collected from fresh produce in the Qatari market

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Abstract

Fruit and vegetable post-harvest infections are mainly caused by mycotoxin producing fungi. Post-harvest diseases are causing food losses that lead to global economical problems. In this study, fresh samples of fruits and vegetables were collected from the local market for shelf-life evaluation and identification of spoiling fungal types. Extracts of three locally available *Prosopis species* leaves were evaluated for their antifungal activity against the sporulation of the isolated fungi. Scanning electron microscopy was also used to evaluate spores and mycelium degenerative changes upon exposure to the leaves extracts. Out of the 156 samples tested, 88.5% showed fungal growth during the 17 days of the experiment. A total of 143 fungi were isolated and were subjected to microscopic identification. The fungal type that was most encountered was *Aspergillus* (30.1%). The fungal spoilage rate was significantly affected by the type of fresh produce ($p \leq 0.05$), while collecting market and country of origin of the samples did not show a significant effect ($p \geq 0.05$). Around 69.5% of the evaluated samples had a moderate to short shelf-life. Ethanolic and aqueous extracts of the leaves of *Prosopis juliflora*, *Prosopis cineraria* and *Prosopis farcta* showed strong effectiveness against fungal sporulation when percent of germinated spores was calculated with alteration in spores and mycelium shapes under SEM. The most effective extract was the ethanolic leaves extract of *P. juliflora*. The three evaluated *Prosopis* spp. extracts showed variation in their effectiveness. A future combination of the most effective crude extracts could be used as a natural bio-controller to replace commonly used chemical anti-fungal agents.

KEYWORDS

bio-pesticide, fungal contamination, leaves extracts, mycotoxins producing fungi, *Prosopis* spp, spoiling agents

1 | INTRODUCTION

Spoilage is the cause of wastage of around 20% of yearly harvested fruits and vegetables around the world. The types of microorganisms and their abundance on fruits and vegetables vary with locations and are affected by: the growth environment, the harvesting conditions, and the handling and storage processes. Freshly harvested fresh produce are prone to contamination with various bacteria and fungi: note that molds are the main causative agent of spoilage mainly in fresh produce refrigerated in open boxes (Mailafia et al., 2017). Fruits and vegetables have high water activity and can be easily contaminated which lead to their spoilable. Contaminated fruits and vegetables are a risk of human health even before the appearance of spoilage as symptoms, many of the contaminating fungi can secrete heat-stable mycotoxins with varied human-health adverse effects (Saleh & Goktepe, 2019). Fungal species that cause fruits and vegetables spoilage include: *Rhizopus, Aspergillus, Penicillium, Cladosporium, Alternaria, Fusarium, Colletotrichum, Pythium, Botritis, Sclerotinia, Phomopsis* and others (Ravichandra., 2021).

Food losses are a worldwide major concern, with the growing world population, food demand increases, and therefore losses limitation and spoilage control become crucial (Quintieri et al., 2021). Commonly used chemical spoilage controllers (antibiotics and fungicides) cause environmental pollution and leave residues that affect human health, which leads many countries worldwide to set maximum residue limits (MRL) of commonly used chemicals on the skin of their imported fresh produce (Saleh & Abu-Dieyeh, 2021). In addition, many spoiling microorganisms are getting resistance to known antimicrobial agents, which put a stress on exploring natural novel products to replace chemicals applied in-field and at post-harvest stage (Bill et al., 2014). A successful naturally based antimicrobial product should eradicate pre-existing infections and leave residues that avoid future infections, it should also retard sporulation of the existing fungal spores and therefore reduces economical losses (Leyva Salas et al., 2017; Sayago et al., 2012; Solanki et al., 2018).

In the Qatari flora, *Mimosaceae* is represented by two genera: the *Acacia* and the *Prosopis*. *Prosopis* is represented by one native endangered species *Prosopis cineraria* known as Al Ghaf and two introduced species: *Prosopis farcta* and *Prosopis juliflora* known as Al Ghuweif. Literature reports that non-native *Prosopis species* were introduced into the area of India and Pakistan around 1878 and has spread since then to other countries. The invasive species *P. juliflora* is now common in the state of Qatar and it is threatening the biodiversity (Abdel-Bari et al., 2007).

According to the Indian council of Forestry Research and Education (ICFRE) *Prosopis spp*. extracts have valuable pharmaceutical values, the flowers extracts have been used for the prevention of miscarriage, while their barks' extracts have shown efficacy in treating asthma, bronchitis, leukoderma, rheumatism, leprosy, and dysentery. Traditionally, eye infections used to be treated by the smoke of *Prosopis* leaves, and leaf extracts were used to treat scorpion and snake bites (Henciya et al., 2017).

Various studies have proved the antimicrobial effectiveness of extracts prepared from *Prosopis* parts. Solanki et al. (2018) have recently discovered a thermostable novel protein extracted from *P. cineraria* pods that showed antifungal activity against *Lasiodiplodia theobromae* and *Aspergillus fumigatus*, the two famous spoiling agents of mango, banana, papaya, strawberry, and orange (Solanki et al., 2018). Powder of leaves, stems and flowers of *P. juliflora* were also proved to be effective in controlling root rot in soil caused by *Fusarium* spp., *Rhizoctonia solani*, and *Macrophomina phaseolina* (Ikram & Dawar, 2013).

The Qatari population has increased by 84.6% between 2008 and 2020. In addition, the prices of fresh produce have increased. This has led to a rise in both the amount imported and the total cost. Starting 2018, Qatar has invested in many agricultural projects to produce some of their fresh fruits and vegetables. However, techniques use are costly compared to other countries with better climate conditions. The total amount of money spent on imported fruits and vegetables in Qatar in 2020 was 1992 MM QR (Planning and Statistics Authority Qatar). These numbers indicate that any loss due to spoilage would cost the country millions, which could have been saved by the development of a safe formulation to increase the shelf-life of fruits and vegetables. This study aims at isolating and identifying the main spoiling agents that affect fresh produce in Qatar and at investigating the anti-fungal effectiveness of the leaf extracts of the three *Prosopis species* available in Qatar. Any successful extract would add to the values of those plants and will open the room for further scientific investigations to develop a stable anti-fungal formulation, which can be used in agriculture and can protect the Qatari environment from the side effects of pesticides.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Fresh, good-looking, imported, and locally grown fruit and vegetable samples were collected from three major markets in Qatar during four trips between September, 2020 and November, 2020. Five replicates were collected from each sample type from a specific country of origin. Collected sample having one country of origin were apple,

avocado, blueberry, capsicum, potato, and cherry-tomato. Samples collected having two countries of origin were carrot, eggplant, grapes, lemon, mango, orange, peach, pear, strawberry, tomato, and zucchini. Whereas, cucumber samples collected had three countries of origin. The total number of samples collected were 155. The samples were handled aseptically and transferred to the laboratory in sterile autoclavable bags with a breathable patch 44 × 20.5 cm (SunBag, transparent; SIGMA-ALDRICH). Data regarding market collection, type, price per Kg, and country of origin for all samples were collected. The collected samples were transferred to the laboratory immediately.

2.2 | Sample processing

All samples were incubated at room temperature, set at 22°C, in separate breathable bags and were observed twice a day for hyphal growth. Upon the first observation of fungal growth, the sample was taken out of the experiment, and the time was noted for the estimation of the shelf-life of the fresh produce. A sterile needle was used to transfer the hyphae for sub-culturing on potato dextrose agar (PDA) (Ham et al., 2016). Different spoiling spot colors and shapes were cultured separately. All PDA plates were incubated until full growth at 25°C. The experiment was terminated at Day 18.

2.3 | Identification of isolated fungi

Mature fungal isolates had their colonies described and were microscopically identified based on their reproductive hyphae and spore morphologies (El Khoury et al., 2006). Lactophenol cotton blue stain was used to identify the isolated fungi. Sterile needles and forceps were used to transfer a part of the fungal mycelia onto a drop of lactophenol cotton blue placed on a clean slide. The wet mount was then covered by a coverslip and the slide was examined at various magnifications with the help of a compound light microscope. The standard taxonomic system was used to classify the isolated fungi based on the characteristics and the morphological description of their conidia and conidiophores (Mailafia et al., 2017).

2.4 | Leaf sample collection and pretreatment

Leaf samples of *P. juliflora*, *P. cineraria*, and *P. farcta* were collected from the field of the Qatar university campus for *P. juliflora* and *P. cineraria* and from Rawdat Al-Faras research station for *P. farcta* after the proper permissions. Leaf samples were transferred to the laboratory in sterile paper bags and were washed thoroughly with tap water followed by sterilized distilled water. The leaf samples were dried at 45°C for 10 days in a pre-sterilized oven. The dried leaves of each *Prosopis spp*. were ground to powder and stored in sterile jars and kept in a cool and dark place until extract preparation (Saleh & Abu-Dieyeh, 2021).

2.5 | Leaves extracts preparation

Twenty grams of each of the *Prosopis* spp. leaf powders were soaked in 200 mL sterile distilled water for aqueous extract preparation and 200 mL of 70% ethanol for ethanolic extract preparation. The leaves were left to soak for 48 h with shaking (50 rpm) at 45°C. The supernatant was then centrifuged to get rid of impurities and was then poured in sterile glass petri plates with covers. The plates were left in an oven at 45°C for the solvents to evaporate. The dried extracts were then scraped from the surface of the petri plates, weighted, and preserved in a refrigerator (4°C). Leaf crude extracts were re-suspended in sterile distilled water to prepare stock solutions, which were sterilized using filter syringes (Sana et al., 2016; Sayago et al., 2012).

2.6 | Crude extract effect on fungal spores' germination

The effect of the six prepared crude extracts was evaluated against the sporulation of 13 out of the 120 identified food-spoiling fungi, which included one strain of *Rhizopus*, *Botrytis*, *Gibberella*, and *Geotrichum*, in addition to two strains of *Fusarium*, three strains of *Penicillium*, and four strains of *Aspergillus*. Test tubes containing 4.5 mL of potato dextrose broth (PDB) were inoculated with 100 µL of spore suspension solution of 10^6 spores/ml. The spores' stock solution was prepared by washing a 7-day-old fungal culture petri dish using 10 mL of sterile distilled water. The spore concentration of the re-collected wash water was determined using a hemocytometer and was to be adjusted to 10^6 spores/ml. The effect of the crude extracts on the germination of spores was determined at four different crude extract concentrations (8, 4, 2, and 1 mg/mL). Tubes with no extracts were also prepared as a negative control. The experiment was conducted in duplicate. Test tubes were incubated at 25°C for 24 h with shaking (150 rpm). Microscopic slides stained with lactophenol cotton blue were prepared in triplicates from each test tube. Out of 100 random conidia, the number of germinated ones was noted, and average germination per test

tube was calculated. A spore is considered germinated if the length of its germination tube reaches at least one-half of the largest dimension of the spore. Spore germination percentages were finally calculated (Zhimo et al., 2016).

2.7 | Spore morphology modifications using a scanning electron microcopy

The spores of fungal strains that were not totally inhibited by any of the crude extracts were tested for their morphological modifications when exposed to the most effective extract. A Spore suspension of 10^5 spores/ml was prepared and exposed to the effective crude extract(s) at the effective concentration. The tubes were incubated at 25°C and 150 rpm for 12 h. The tubes were then centrifuged (2800 *g*), and the pellet was washed twice with PBS pH 7.4. The pellet was re-eluted in a solution of 2.5% glutaraldehyde +3.6% of formalin and was incubated at 4°C for 18 h. The tubes were then centrifuged (2800 *g*) and washed three times with PBS. The pellets were dehydrated in different ethanol dilutions (25%, 50%, 70%, 80%, 90%, and 100%) for 30 min each. The samples were then smeared on silver holders in thin films and left to air dry before gold coating them using agar sputter coater. The SEM observations were made with Nova NanoSEM 4503 (Saleh & Abu-Dieyeh, 2021).

TABLE 1 Average extraction yield (%).

	Prosopis juliflora	*Prosopis cineraria*	*Prosopis farcta*
Aqueous extract	12.75	9.9	12.75
Ethanolic extract	19.15	10.9	17.95

2.8 | Statistical analysis

Data were analyzed using SPSS statistical software (Ver. 27, SPSS Inc. Chicago, USA). The Chi-square test was used to evaluate the effect of samples type, collection market, and commodities' country of origin on the detection of fungal spoiling agents at a significance level $p \leq 0.05$.

3 | RESULTS AND DISCUSSION

3.1 | Plant extract yield

Upon the preparation of aqueous and ethanolic extracts of *P. juliflora*, *P. cineraria*, and *P. farcta*, total final weights of scratched extracts obtained from each 20 g of leaves were recorded and extraction yields were calculated as percent final extract weight out of the 20 g leaves powder (Table 1).

It can be noticed that the ethanolic extraction method has a higher yield than the aqueous extraction method. In addition, *P. cineraria* showed a lower extraction yield compared to the two other *Prosopis species*. A previous study on aqueous and ethanolic extracts of a pharmaceutically rich plant, *Withnaia somnifera*, *showed that different extractions methods gave different extract yields with an average yield of aqueous extracts of 9.5%*, *which was close to our yield of P. cineraria extract* (Dhanani et al., 2017). The usage of the same extraction method and solvent gives different extraction yields for different plants depending on the amounts of the available soluble phytochemicals in this plant. Different solvents also give different extraction yields for the same plant, which also indicate the variation of soluble phytochemicals. Four different solvents were used for extract preparation

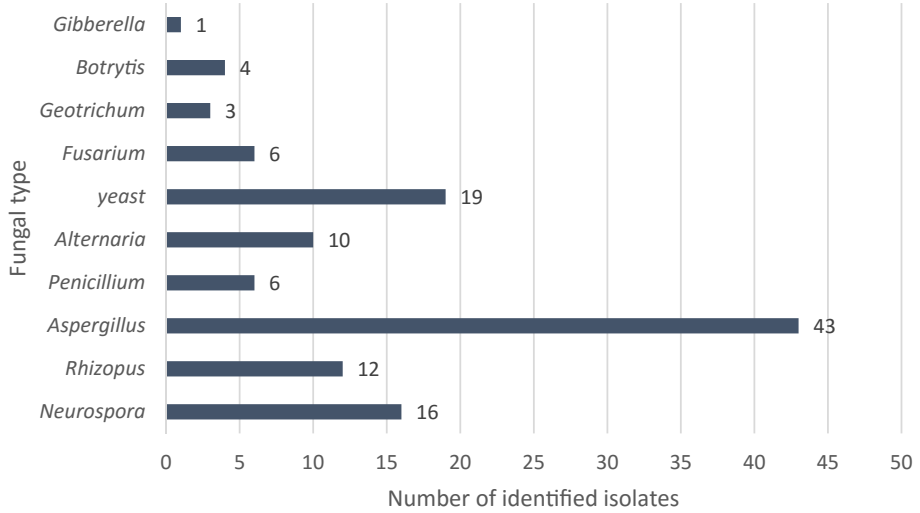


FIGURE 1 Numbers of identified fungi isolated from different types of fruits and vegetables.

TABLE 2 Type of fungi isolated from every main market in the study and the ratio of number of fungal isolates versus number of samples collected.

	Neurospora	*Rhizopus*	*Aspergillus*	*Penicillium*	*Alternaria*	*Yeast*	*Fusarium*	*Geotrichum*	*Botrytis*	*Gibberella*	Unidentified	Total	Ratio of isolates versus samples collected
Market 1	15	6	19	3	6	8	2	2	0	1	3	65	**1.07**
Market 2	2	0	7	2	4	5	1	1	0	0	2	24	**0.96**
Market 3	0	4	8	1	0	6	3	0	4	0	17	43	**0.72**

TABLE 3 After purchase average shelf-life of fresh commodities purchased with different countries of origin.

Sample type	Country of origin	Shelf-life (days)
Cucumber	Iran	3
	Qatar	11.5
Grapes	Lebanon	8.2
	Turkey	14.8
Mango	Indonesia	12.2
	Kenya	15.2
Orange	South Africa	12.6
	Australia	8.8
Pear	Lebanon	6.6
	Turkey	8.8
Zucchini	Qatar	13.2
	Turkey	4.2

from the bark of *Azadirachta indica*, and the leaves of *Acalypha wilkesiana* and *Solanum scabrum* showed average extraction yields between 2.73% and 17.23% (Anokwuru et al., 2011).

3.2 | Isolated fungi

Out of the 155 samples tested, 88.4% showed fungal growth within the 17 days of the experiment, and various types of fungi were isolated from different fruits and vegetables. A total of 143 fungi were isolated and were subjected to microscopic identification. The most encountered fungal type was *Aspergillus* (30.1%), followed by yeast (13.3%) and *Neurospora* (11.2%). Different mycotoxin producing fungi were isolated in the study (Figure 1).

Aspergillus is a common fungi that is easily transmitted by air, which explain its high occurrence among the samples. A previous study conducted on fresh produce fungal contamination in Qatar showed *Penicillium* as the most encountered contaminant followed by *Rhizopus*: the difference between the previous surveillance and the current study is mainly in the commodities' types. Oranges were around 25% of the total number of samples in the previous study, and they were mainly contaminated with *Penicillium*, which explains the difference (Saleh & Al-Thani, 2019). A study conducted in the Washington D.C. area on nuts and dried fruits was similar to our current study with *Aspergillus* being the most common isolate among a total of 117 identified fungi (Tournas et al., 2015). Similarly, a study conducted in Lebanon on grapes showed high levels of contamination with different strains of *Aspergillus* (El Khoury et al., 2006).

Studies conducted to determine fungal contamination levels in food products in the Gulf region are few. A study conducted in Bahrain to evaluate contamination levels of 17 types of imported spices, showed *Aspergillus* as the most encountered fungi followed by *Penicillium* (Mandeel, 2005). A study on 520 date samples in Saudi Arabia showed *Penicillium* and *Cladosporium* as the two most isolated fungi (Hamad, 2008). Despite the variation in samples types, both studies results showed harmony with the current results.

3.3 | Sample contamination rates

The Chi-square test demonstrated that the detection of fungal spoilage was significantly different among various fresh produce types ($p = 0.001 \leq 0.05$). The water content levels are different among the various sample types chosen. Furthermore, the nutritional facts of the samples types are different. Therefore, different spoiling agents showed correlation to specific fresh produce. Out of the 18 samples that did not show any fungal growth during

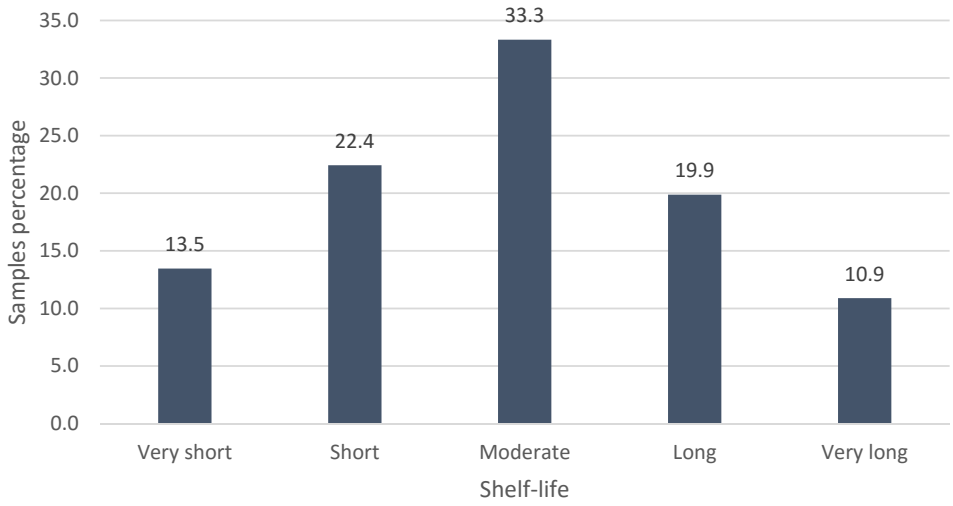


FIGURE 2 Percentage of fresh produce samples having a certain shelf-life. Very short shelf-life: the sample showed fungal growth between 1 and 3 days. Short shelf-life: the sample showed fungal growth between 4 and 7 days. Moderate shelf-life: the sample showed fungal growth between 8 and 11 days. Long shelf-life: the sample showed fungal growth between 12 and 16 days. Very long shelf-life: the sample did not show fungal growth up to 17 days.

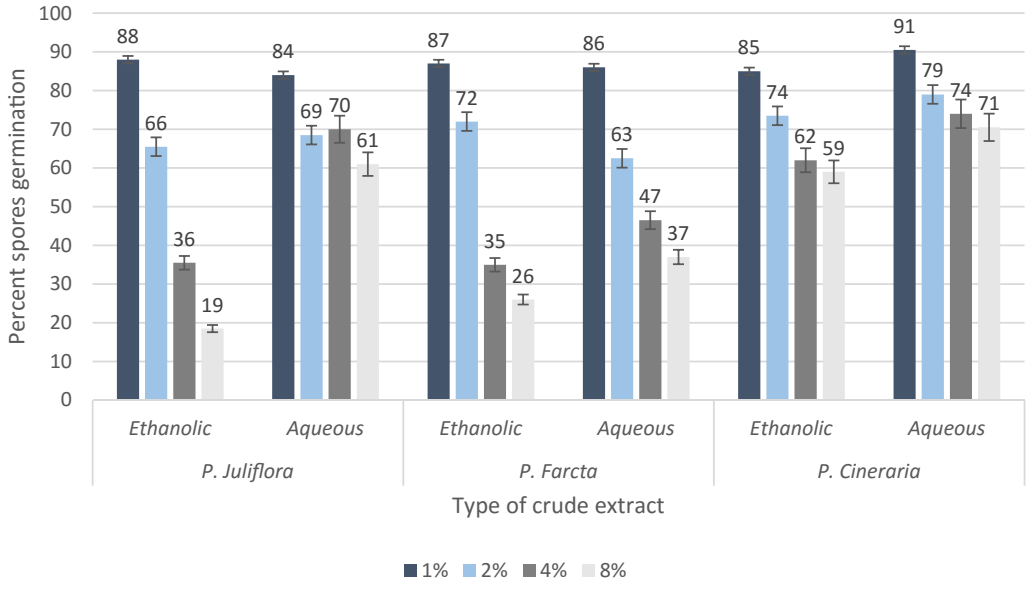


FIGURE 3 Percentage of germinating *Botrytis* spores after 24 h of exposure to different concentrations of *Prosopis* leaf crude extracts at 25°C with shaking (150 rpm).

TABLE 4 Percentage of germinating spores after 24 h of exposure to 8 mg/mL of *Prosopis* leaves crude extracts at 25°C with shaking (150 rpm).

		Botrytis	*Rhizopus*	*Gibberella*	*Geotrichum*	*Fusarium* 1	*Fusarium* 2	*Penicillium* 1	*Penicillium* 2	*Penicillium* 3	*Aspergillus* 1	*Aspergillus* 2	*Aspergillus* 3	*Aspergillus* 4
Prosopis juliflora	Leaves ethanolic extract	19	15	34	22	51	32	38	16	38	59	37	32	32
	Leaves aqueous extract	61	50.9	76	62	61	79	60	21	66	55	37	24	25
Prosopis farcta	Leaves ethanolic extract	26	22	47	25	45	28	48	23	48	70	37	28	32
	Leaves aqueous extract	37	22.7	39	25	31	80	31	18	31	40	40	13	12
Prosopis cineraria	Leaves ethanolic extract	59	72.5	52	35	42	13	34	23	34	53	32	14	41
	Leaves aqueous extract	71	24.5	73	36	25	70	27	41	27	34	55	24	67

the experiment, ten samples are lemon samples which are known for having a long shelf-life (Artés-Hernández et al., 2007). All *Neurospora* isolates were taken from pear and peach samples. In addition, all *Botrytis* identified were isolated from strawberry samples, which was consistent with studies indicating *Botrytis* as the main spoiling agent of strawberries (Abdelfattah et al., 2016; Hassan et al., 2021).

3.4 | Effect of displaying market on fresh produce spoilage

Samples were mainly collected from three large supermarkets in Doha. Table 2 shows the type of fungi isolated from each of the three main markets in the study and the ratio of number of fungal isolates versus number of samples collected from each market.

Although, Market 1 showed the highest ratio, Chi-square test results showed that the number of samples showing fungal spoilage within 17 days was not significantly different among samples collected from different markets ($p = 0.148 \geq 0.05$). Although certain fungal types are more concentrated in specific markets, yet no market-related conclusions can be made, as the fungal isolates are more likely to be sample-related knowing that different samples were collected from different markets. The results show that the storage and displaying practices used in different markets in the country did not affect significantly the transfer of fungi on fresh produce.

3.5 | Effect of fruits country of origin on fruit spoilage

When testing for the effect of the country of origin on commodities' fungal spoilage. Chi-square test showed significance with $p = 0.13 \geq 0.05$. However, different commodities were purchased from different countries of origins, which make the comparison biased. Similar results were shown in a previous study conducted in Qatar, where statistical analysis did not show a difference in the microbiological quality of fresh produce samples collected from developed and developing countries (Saleh & Al-Thani, 2019). A more logical comparison is represented in Table 3, where the shelf-lives in days of similar commodity types purchased with different countries of origin are represented.

It can be concluded from the cases of zucchini and cucumber that the locally grown fresh produce have a longer shelf-life, which is logical as they are being made available in the market freshly after harvesting. Although differences are obvious in many of the cases, more samples are

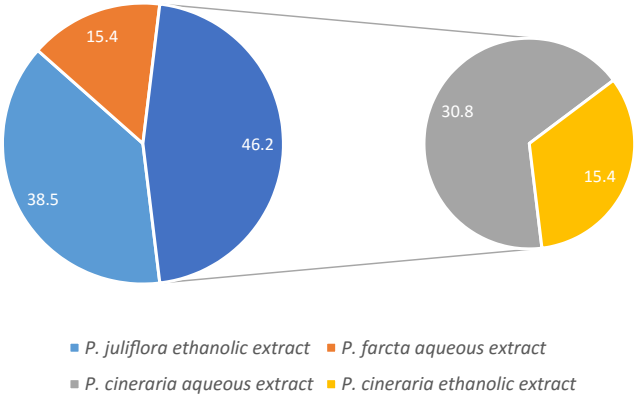


FIGURE 4 Frequency at which a crude extract is the most effective within a trial out of the 13 trials.

needed in future studies in order to conclusively relate the shelf-life of fresh produce to the country of origin. In addition, it is critical to make sure that commodities compared belong to the same type, as certain genetically modified species or pesticides coated samples can last much longer than organic products, yet their nutritional values will be questionable (Bhatia et al., 2020). Samples of strawberry and eggplants showed short shelf-life regardless of their country of origin. Similarly, samples of pears and tomatoes from different origins showed moderate shelf-life. Finally, all lemon samples used did not show any fungal growth during the experiment regardless of their country of origin.

3.6 | After purchase shelf-life of various commodities

The time taken by a sample to show fungal growth varied from one to more than 17days. Based on the rotting speed, samples' shelf-lives were divided into the below categories:

1. Very short shelf-life: the sample rotten between 1 and 3 days.
2. Short shelf-life: the sample rotten between 4 and 7 days.
3. Moderate shelf-life: the sample rotten between 8 and 11 days.
4. Long shelf-life: the sample rotten between 12 and 16 days.
5. Very long shelf-life: the sample did not show fungal growth up to 17 days.

Figure 2 shows the percentage of samples having long, moderate, or short shelf-life.

Strawberry samples had the shortest shelf-life, with many samples rotting within 3 days. Similarly, a previous study conducted in Qatar showed the shortest shelf-life for strawberry samples (Saleh & Al-Thani, 2019). Eggplants and peach showed also short shelf-life of 3 days and 4.4 days, respectively. Lemon samples showed the longest shelf-life as previously described.

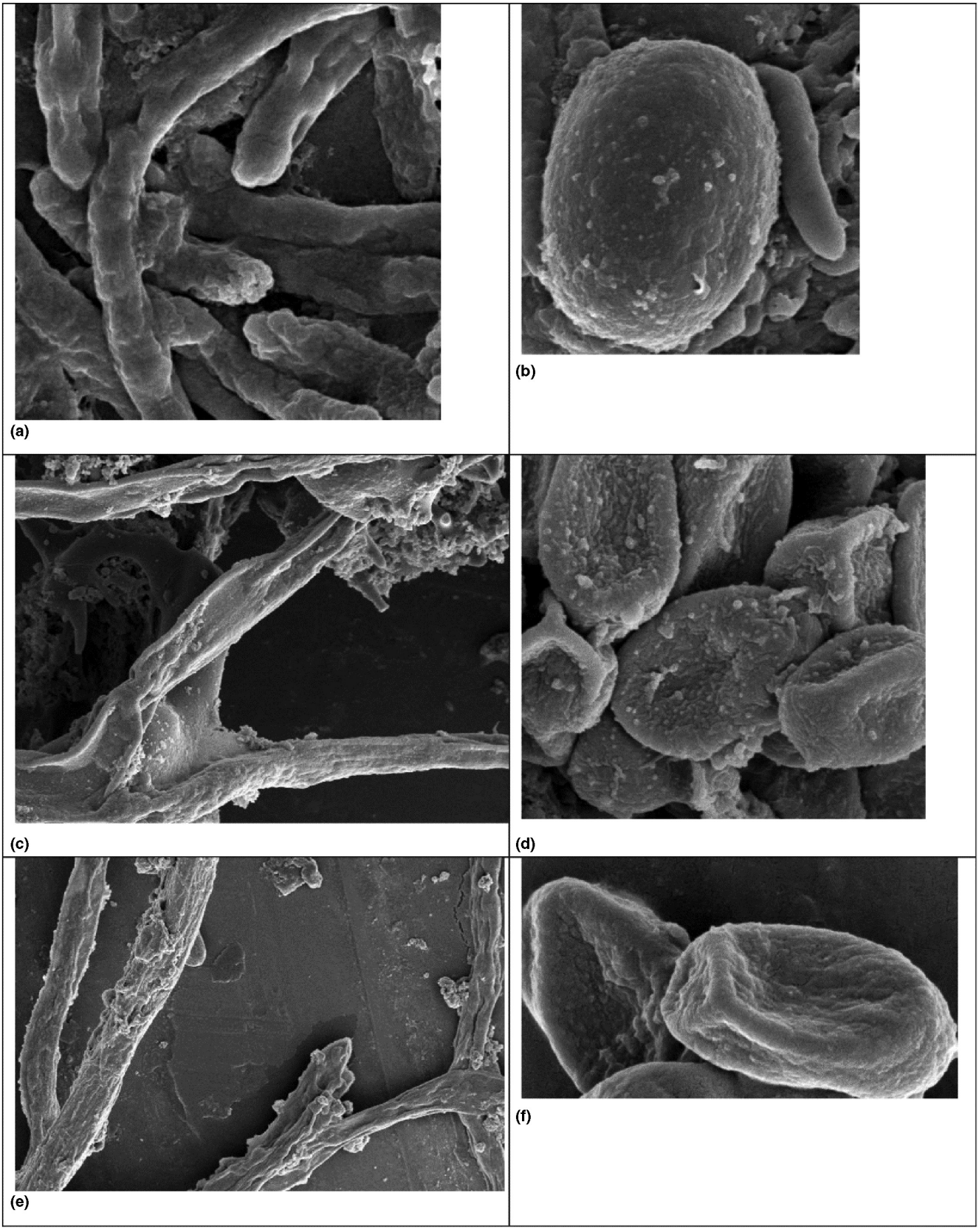
This surveillance has demonstrated a high level of mycotoxins' producing fungi in daily-consumed food products. Implementation of safety programs such as Hazard Analysis and Critical Control Point (HACCP) in food production industry and the application of good agricultural practices (GAP) would lower contamination levels (Cusato et al., 2014; Gomes et al., 2014). However, spoilage controller applications is still necessary, which emphasis the importance of biological controllers development (Nešić et al., 2021).

3.7 | Crude extracts effect on fungal spores' germination

When *Botrytis* had its spores, their sporulation was tested upon exposure to the six crude extracts. The results showed that the most effective extracts was the ethanolic leaf extracts of *P. juliflora*, lowering the percent germination to 19% at 8 mg/mL, followed by *P. farcta* lowering the percent germination to 26% at 8 mg/mL (Figure 3).

Botrytis results are consistent with previous experiments conducted in the same lab showing lowering of *Botrytis* germination percentage in the presence of *P. juliflora* leaves ethanolic extract (Saleh & Abu-Dieyeh, 2021).

FIGURE 5 Scanning electron microscope micrographs of hyphae and spores of control fungi and treated fungi with 8 mg/mL concentration extracts. (a) *Penicillium* hyphae non-treated (20,000x). (b) *Penicillium* spore non-treated (20,000x). (c) *Penicillium* hyphae treated with *P. juliflora* leaf ethanolic extract (5.000x). (d) *Penicillium* spores treated with *P. juliflora* leaf ethanolic extract (20,000x). (e) *Penicillium* hyphae treated with *P. cineraria* leaf ethanolic extract (2.500x). (f) *Penicillium* spores treated with *P. cineraria* leaf ethanolic extract (20,000x). (g) *Penicillium* hyphae treated with *P. farcta* leaf ethanolic extract (20,000x). (h) *Penicillium* spores treated with *P. farcta* leaf ethanolic extract (20,000x). (i) *Gibberella* hyphae non-treated (5.000x). (j) *Gibberella* spores non-treated (10,000x). (k) *Gibberella* hyphae treated with *P. juliflora* leaf ethanolic extract (5.000x). (l) *Gibberella* spores treated with *P. juliflora* leaf ethanolic extract (5.000x). (m) *Gibberella* hyphae treated with *P. cineraria* leaf ethanolic extract (5.000x). (n) *Gibberella* spores treated with *P. cineraria* leaf ethanolic extract (20,000x). (o) *Gibberella* hyphae treated with *P. farcta* leaf ethanolic extract (5.000x). (p) *Gibberella* spores treated with *P. farcta* leaf ethanolic extract (10,000x).



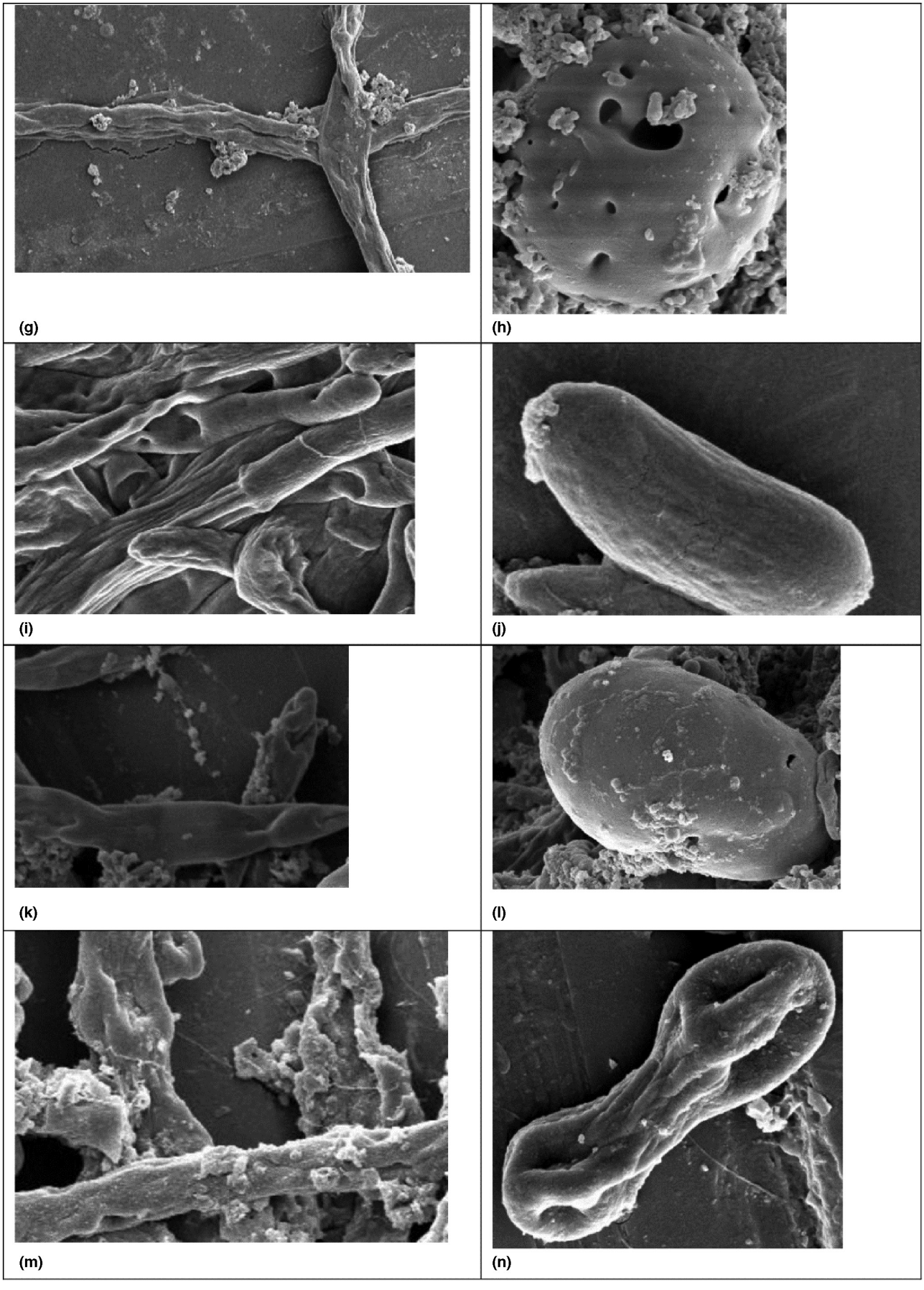


FIGURE 5 (Continued)

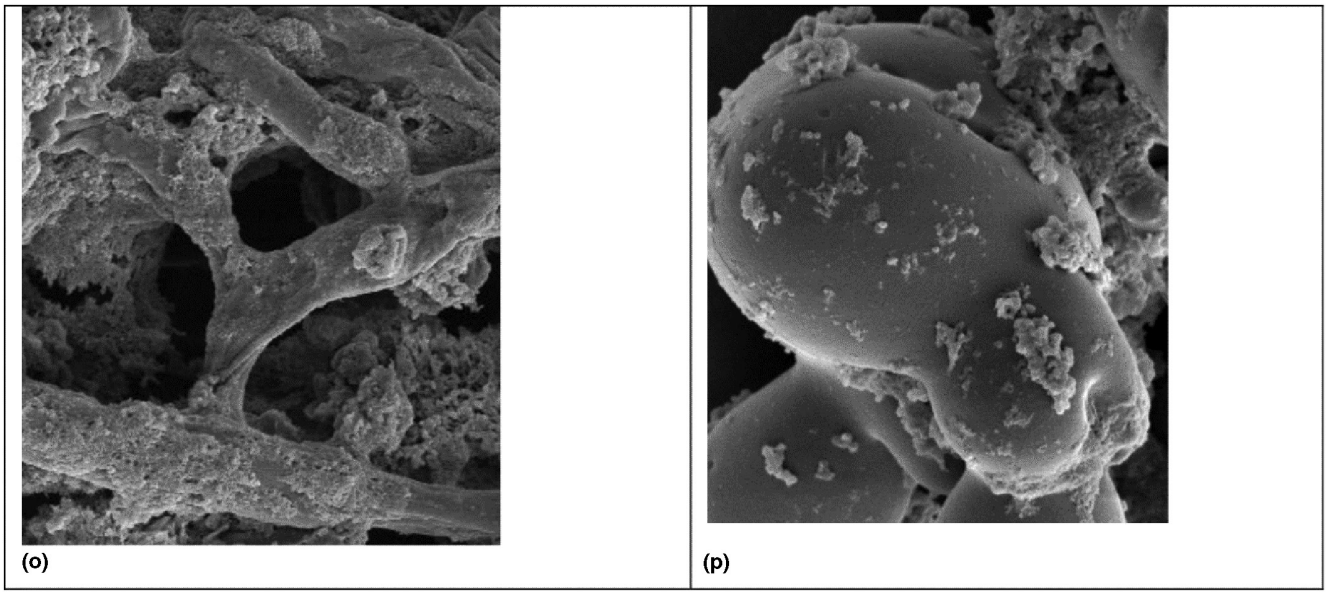


FIGURE 5 (Continued)

Control tubes without plants extracts showed 98%–100% germination rate. As Figure 3 shows a dose dependent germination percent decrease with the increase in the six crude extracts concentrations, the results were summarized as percent germination at 8 mg/mL of each crude extract (Table 4).

The least affected fungi were: *Gibberella*, *Aspergillus* strain 1, *Aspergillus* strain 2, and *Penicillium* strain 1. The most effective extract was the ethanolic leaves extract of *P. juliflora*, this is in consistence with previous results obtained in the lab which proves the strong effectiveness of this extract against spores, germination, although the percent germinations showed some differences that could be due to the different species tested (Saleh & Abu-Dieyeh, 2021). The current anti-sporulation results are supported in many studies about *Prosopis species* around the world. Literature shows the strong antimicrobial effectiveness of *P. juliflora*, *P. cineraria* and *P. farcta* leaves' ethanolic and aqueous extracts have (Jameel et al., 2019; Salari et al., 2019; Thakur et al., 2014). Interestingly, other species of *Prosopis* showed effectiveness against fungal strains that were not affected by *P. juliflora*, which indicates variation in the effective phytochemicals. Figure 4 shows the frequency of each of the six crude extracts being the most effective extracts within the 13 trials.

Six of the tested strains had their sporulation percentage lowered to below 20%, increasing the concentration of the extract might lead to total eradication of the fungi, and even the species that were not totally killed had their growth lowered, which indicated that the extracts would, when applied on fruits and vegetables, slow down fungal growth and extent shelf-life.

3.8 | The effect of *Prosopis* extracts on the microscopic morphology of two fungal species using a scanning electron microscope (SEM)

Two of the least affected fungi (*Penicillium* and *Gibberella*) had their morphology evaluated using SEM upon exposure to the ethanolic extracts of the *Prosopis* leaves. *Penicillium* treated with *P. juliflora* leaf ethanolic extract (Figure 5c) showed hyphae applanation, shrinkage and flake formation compared to the control (non-treated) hyphae (Figure 5a). *Penicillium* spores treated with the same extract are collapsed (Figure 5d) compared to the normal shape of the control spore (Figure 5b). Clear degenerative changes were seen upon the treatment with *P. cineraria* leaves ethanolic extracts where hyphae showed a loss of smoothness (Figure 5e) and spores totally collapsed (Figure 5f). Treatment of *Penicillium* with *P. farcta* leaves ethanolic extract has lead to the same flat, non-tubular mycelium (Figure 5g) with spores showing different sizes of pores that would lead to leakage of essential intracellular components (Figure 5h).

Non-treated *Gibberella* showed extensive net of hyphae, that have fine tubular morphology with smooth spores (Figure 5i,j), while after treatment with *P. juliflora* leaves ethanolic extract the hyphae lost its consistency in shape and got more applanate (Figure 5k), and pores formation can be noticed on spores with intracellular excretions (Figure 5l). *Gibberella* treated with *P. cineraria* leaves ethanolic extracts showed pores and secretion in intracellular substances in hyphae (Figure 5m) with totally collapsed spores (Figure 5n). Treatment of *Gibberella* with

P. farcta leaf ethanolic extract led to formation of exfoliated flakes on hyphae outer membrane (Figure 5o) with partial spores collapsing and pore formation (Figure 5p).

Similar effects were seen using SEM when various strains of fungi were treated with *P. juliflora* leaves ethanolic extract in a previous study by the same lab team (Saleh & Abu-Dieyeh, 2021). Although the inhibition of sporulation of fungi was not complete and although extracts effectiveness on Gibberella and Penicillium was low, yet the morphological changes and the damages in hyphae and spores indicate that the extracts might have good effect in delaying or disrupting fungal growth on fresh produce and therefore protecting food from fungal spoilage.

4 | CONCLUSION

The determination of the most common spoiling agents in fruits and vegetables in the local market is of major importance to be able to well plan the anti-fungal treatment. Such surveillance analysis should be repeated every season for better conclusive results. Having continuously lower fungal isolates ratios versus collected samples is an indicator that encourages investigations into the best hygienic practices adopted by the market.

The anti-sporulation experiment shows very promising results, a future combination of the most effective crude extracts could replace commonly used anti-fungal agents to save the environment and could extend fresh produce storage life and decrease economic losses. Even the least affected fungal strains had their morphology affected when tested under SEM, which proves extracts effectiveness. *P. juliflora* ethanolic leaves extracts was the most effective, however, species that was not affected at all by this extract were affected by other extracts such as *P. cineraria* extracts and *P. farcta* ethanolic extract. Therefore, the effect of combination of all effective extracts is worth trying as a wide spectrum bio-pesticide.

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CONFLICT OF INTEREST STATEMENT

The authors have stated explicitly that there are no conflict of interest in connection with this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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