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Growth Inhibition of Phytopathogenic *Penicillium citrinum* and *Penicillium expansum* by Some Indian Culinary Spices

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Abstract

Penicillium citrinum and *Penicillium expansum* are primarily associated with the spoilage of wide variety of fruits and derived food products. Increasing demand of consumers for preservative (synthetic) free food has led the researchers to explore natural substances for controlling spoilage and pathogenic microbes. Past researches have demonstrated the antimicrobial activities of spices. Present study was undertaken to assess the growth inhibitory activities of aqueous extracts (AEs), essential oils (EOs) and powdered (PD) forms of 10 spices towards *P. citrinum (MTCC2553)* and *P. expansum (MTCC2006)*. PD spices were evaluated for their growth inhibitory potential using spice agar method. Impregnated paper disc method was followed for the antifungal screening of AEs and EOs, while broth dilution method was opted for the determination of their minimum inhibitory concentrations. Results revealed that AEs of all the spices were found ineffective, whereas PD forms of three spices, namely, *Cinnamomum cassia (Blume), Cuminum cyminum* and *Syzygium aromaticum,* significantly arrested the growth of both the fungal strains. Nevertheless, EOs of *Allium sativum, Brassica juncea, C. cassia (Blume), C. cyminum, Mentha piperita, Ocimum sanctum and S. aromaticum,* exhibited remarkable antifungal activities against both the fungi. *P. citrinum* was more susceptible as compared to *P. expansum,* towards tested substances. According to our results, PD spices, being cheap and safe, may be persued as 'green antimicrobials' along with spice EOs, for *in vivo* studies to extend the shelf life of fruits and their processed products. Therefore, this study would prove a great help to the agricultural sector and food processing industry.

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Introduction

Fruits are perishable by nature and their low pH values give fungi a competitive advantage over most bacteria and yeasts, thereby making them more prone to fungal onslaught. Among the most encountered species of fungi responsible for the spoilage of fruits and derived products, belong to the genus *Penicillium.*[1]

A novel emerging fungal pathogen often seen on moldy citrus fruits, which hails from the above stated genus is *Penicillium citrinum*.[1,2] It has been isolated from cucumber, dried wine fruits, grapes, lemon, oranges, persian lime, sweet lime and tomatoes.[2-6] Occurrence of *P. citrinum* has also been reported on *capsicum* sp. during pre-harvest, harvest, and post-harvest stages,[7] where it serves as the predominant source of mycotoxin citrinin, which poses serious health concerns and economic threat, globally, due to its potential hepatotoxicity and nephrotoxicity to humans and adverse impact on valuable agricultural commodities.[8]

Another broad spectrum fungal pathogen is *Penicillium expansum,* and it is the only *Penicillium* species that can express pathogenicity over a variety of fruits, viz., apricots, avocadoes, citrus, grapes, kiwi fruits, mangoes, nectarines, plums, pome fruit, stone fruit, strawberries and tomatoes.[9,10] *P. expansum* is also well known for causing the rotting of apples and pears, and is mainly responsible for the production of mycotoxin patulin in juices extracted from the rotted fruits.[11,12] Consumption of patulin contaminated food products may lead to various health hazards in humans involving gastrointestinal disorders, nausea and pulmonary congestion along with possible carcinogenic, genotoxic, immunotoxic, neurotoxic and teratogenic effects.[13]

Till date, use of synthetic chemicals is prevalent to control the growth of microbes for the preservation of raw agricultural commodities and processed food products, which is continuously being challenged by consumers and scientific community due to their possible toxicity, harmful effect on environment, high cost and development of resistant races of pathogens.[14] Hence, there is an increasing demand of natural preservatives, those are safe, cost effective and eco-friendly.[15,16] Spices being of plant origin, undoubtedly, lie under this category.

India is the world's largest consumer, exporter, and producer of spices.[17] Despite the fact that use of spices for the preservation of food products dates back history, these precious horticultural crops are widely being rediscovered for their antioxidant and antimicrobial properties, for last few decades. Although, various findings have been reported on the antimicrobial activities of spice extracts and some spice phtyochemicals, such as allicin, cinnamic aldehyde and eugenol etc., towards several pathogenic microbes of animals and humans (*Aspergillus* spp., *Bacillus* spp., *Enterococcus faecalis, Escherichia coli, Listeria monocytogenes, Pseudomonas spp., Salmonella spp., Shigella sonnei, Staphylococcus aureus, Vibrio parahaemolyticus* and many others).[18-23] It has also been well documented that EOs and their components bind with the lipid bilayer of the bacterial cell membrane and mitochondria, and alter the cell structures.[23] But, there is scanty information available on the inhibitory effect of spices on fungi causing the spoilage of fruits and vegetables.

Considering above aspects in mind, present study was designed to investigate growth inhibitory potentials of AEs, EOs and PD forms of some Indian culinary spices, towards phytopathogenic *Penicillium citrinum (MTCC2553)* and *Penicillium* expansum *(MTCC2006)*. In comparison to previous researches, which mainly focused on antimicrobial properties of spice EOs, we screened PD spices as well, because spices in their later form are most commonly used in day-to-day domestic culinary practices in India as an important seasoning ingredient. Secondly, PD spices cost less as compared to their corresponding EOs, those involve expensive and tedious methods of production.

Therefore, this study would serve as harbinger to establish spices as 'green antimicrobials' to extend the shelf life of fruits and vegetables and derived products, which would be contributing to agricultural sector and food processing industry, equally.

Materials and Methods

Procurement and Preparation of Spice *Sa*mples

PD spice *sa*mples

Plant parts of *Allium sativum (As)*, *Mentha piperita (Mp) and Zingiber officinale (Zo),* were procured in their fresh forms, from a local grocery shop.

Ocimum sanctum (Os) leaves were hand plucked from a home grown plant. Peels of *As* bulbs and Zo rhizomes were removed manually with knife. Fresh forms of aforementioned 4 spices were washed with distilled water to remove the extraneous matter, and were subsequently dried under ambient conditions in shade.

Dried plant parts of *Brassica juncea (Bj), Cinnamomum cassia (Blume) (Ccb), Cuminum cyminum (Ccm), Curcuma longa (Cl), Syzygium aromaticum (Sa) and Trigonella foenum-graecum (Tf-g)* were purchased from a spice wholesaler of local market, and were manually cleaned to remove extraneous material.

Dried forms of spices thus obtained, were ground to powdered (PD) forms with the help of a grinder in the laboratory and were stored in hermatically sealed jars till their further use.

Botanical features of Indian culinary spices used in current study are presented in Table 1.

EOs of Spices

EOs of spices were purchased from Aroma Chemicals Pvt. Limited, India. Company assured the purity of the spice EOs to be more than 98.0 %.

AEs of Spices

Spice AEs were prepared by the method described elsewhere.[21] All the AEs were collected in sterilized glass vials, and were further used within 2 h. of their preparation, at various concentration levels as per the requirement of experiment.

Table 1: Botanical features of Indian culinary spices used in current study

Botanical names	Indian names	English names	Family	Plant parts of spices used
Allium sativum L.	Lehasun	Garlic	*Liliaceae*	Bulbs
Brassica juncea L.	Sarson	Brown Mustard	*Crucifereae*	Seeds
Cinnamomum cassia (Blume)	Dalchini	Cinnamon	*Lauraceae*	Bark
Cuminum cyminum L.	Jeera	Cumin	*Apiaceae*	Seeds
Curcuma longa L.	Haldi	Turmeric	*Zingiberaceae*	Rhizomes
Mentha piperita L.	Paudina	Mint	*Labiateae*	Leaves
Ocimum sanctum L.	Tulsi	Holy Basil	*Lamiaceae*	Leaves
Syzygium aromaticum L.	Laung	Clove	*Myrtaceae*	Flower Buds
Trigonella foenum-graecum L.	Methi	Fenugreek	*Leguminosae*	Seeds
Zingiber officinale L.	Adarak	Ginger	*Zingiberaceae*	Rhizomes

Fungal Strains and Growth Conditions

Pure cultures of *Penicillium citrinum (MTCC 2553)* and *Penicillium* expansum *(MTCC 2006)* were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India Potato dextrose broth medium (PDB) and Potato dextrose agar medium (PDA) (Hi Media, India) were used for the cultivation of both the fungi (temperature of incubation: 25° C ; duration of incubation : 48 h-96 h), as per the recommendations of MTCC. Initially, the growth of both the fungi appeared as white mycelium on PDA that turned to greenish to bluish green in colour as the spores matured.

Preparation of Fungal Inoculum

For the preparation of inoculum, bluish green spores of *P. citrinum* and *P. expansum* were harvested from 15 days old slants by adding 0.05% of Tween 80 ((Central Drug House (CDH), India)). These harvested spores were quantified by a haemocytometer to adjust at 10^7 spores/mL.

Screening Inhibitory Activities of Spice *Sa*mples Spice Agar Method

Spice agar method[24] was used for the determination of inhibitory potentials of PD spice *sa*mples. Freshly prepared fungal inoculum (100 µL) was

evenly spread over the surface of solidified PDA petri plates previously supplemented with various concentrations of PD spices (0.1 - 5.0 (%,w/v)). Seeded petri plates were incubated at 25°C for 30 days (total incubation period), and were examined for the growth of fungi at a regular interval of 12 h., consistently. The time for the initiation of fungal growth on petri plates were recorded. A control set of experiment (without any PD spice *sa*mple), was also conducted, parallelly.

From the results of spice agar method, minimum inhibitory concentrations (MICs) of PD spice *sa*mples were derived. For the *sa*ke, concentrations of spices (C*OS* (%, w/v)) were plotted on the x-axis and days of inhibition (DOI) were plotted on the y-axis of graph (Figure 1). Then, 40%, 60% and 80% levels of total incubation period (30 days), were calculated. Afterwards, from each referred level, a horizontal line was drawn to intersect the curve. Subsequently, a perpendicular line was drawn from the point of intersection, which corresponded to the concentration of spice *sa*mple. MIC was the concentration of PD spice *sa*mple which did not allow the fungi to grow up to 80% level, i.e., 24 days.

Impregnated Paper Disc Method

Impregnated paper disc method, narrated by someone else[25] was opted for screening antifungal activities of AEs and EOs of spices. Results were presented as net zones of inhibition (mm) after subtracting diameter of filter paper discs (6mm) from the diameter of measured inhibitory zones.

Broth Dilution Ass*a*y

Broth dilution as*sa*y,[25] was used to determine minimum inhibitory concentrations (MICs) of AEs and EOs (which exhibited distinct inhibitory zones during impregnated disc method). PDB containing 2000 µL/mL of test EOs were serially diluted two fold each with PDB to get concentration levels of 1000, 500, 250, 125, 62.50, 31.25, 15.62, 7.81, 3.90, 1.95, 0.97, 0.48, 0.24, 0.12, 0.06 µL/mL. Then, 100 µL of inoculum of test fungi was added to them. Mixtures thus obtained were incubated at 25ºC for 96 h. After the stipulated incubation period, 100 µL of above mixture was spread on the surface of solidified PDA plates. The later were incubated for 96 h, at 25 ºC to notice the lowest dilution of test *sa*mples, which did not show any visible growth of fungi.

*During impregnated paper disc method and broth dilution as*sa*y, dimethylsulphoxide (DMSO) (CDH, India), was used as a negative control agent. All the above experiments were conducted thrice, simultaneously.

Statistical Analysis

Results of zone inhibition as*sa*y were statistically analyzed by using SPSS version 7.5 and were expressed as the mean ± standard deviation.

Results

Inhibitory Activities of PD spices

Data presented in Table 2 indicate that PDA petri dishes incorporated with PD forms of *Ccb*, *Ccm* and *Sa*, at their different concentration levels (from 0.1 to 5.0 (%,w/v)), potentially inhibited *P. citrinum* (*Pcit*) and *P. expansum* (*Pexp*). On the contrary, *Bj, Cl, Mp, Os, Tf-g* and *Zo*, up to their highest concentration level of 5.0% (w/v), were found ineffective in inhibiting test fungal strains, and their growth in culture media in the form of white mycelium was observed on the 2nd day of incubation as in control set of petri dishes without any PD spice *sa*mple. However, *As* displayed very weak inhibitory effect only towards *Pcit*, and former at 5.0% level, delayed the growth of later by 4 days. It is obvious from the data of spice agar method that both the fungi responded in their own peculiar manner towards various concentrations of *Ccb*, *Ccm* and *Sa*. *Ccb* and *Sa* at a concentration level as low as 0.1% (w/v), exerted their inhibitory effects towards *Pcit*, while growth of *Pexp* was delayed by both the aforestated spices at their 0.2% levels (w/v).

It was also noticed that number of days of inhibition increased with the increase in the concentration of PD spices. And, *Sa* at concentration levels of 2.0% (w/v) and 3.0% (w/v) delayed the growth of *Pcit* and *Pexp* respectively, by 30 days. Similarly, *Ccb* at 3.0% (w/v) level, hindered the visible growth of both the test fungi throughout the incubation period. On the other hand, *Ccm* up to 1.5% (w/v) was found to be ineffective towards test fungi, and showed its inhibitory effect at a concentration level of 2.0% (w/v) by impeding the growth of *Pcit* and *Pexp* for 5 days and 4 days, respectively. At 5.0% (w/v) level, *Ccm* delayed the visible growth of *Pcit* up to 21 days, and that of *Pexp* up to 17 days, and was thus unable to produce the desired growth inhibitory effect even at its highest stated concentration level.

It is worth mentioning here that different levels of inhibition were generated by different concentrations of referred spices towards both the microbes under question (Figure 1, Table 3). *Ccb* and *Sa* produced 40%, 60% and 80% levels of inhibition towards both the fungal strains, while in case of *Ccm*, 80% level of inhibition was not detected. Though, a positive and direct relation was observed between different levels of inhibition generated and the concentration of PD spices used (Figure 1). The concentration of spice which produced 80% level of inhibition against test fungi was considered as minimum inhibitory concentration (MIC) (Figure 1, Table 3). It is quite noteworthy that lower concentrations of PD spices were required to produce any given level of inhibition against *Pcit* as compared to *Pexp*.

On the basis of days of inhibition produced and MIC values obtained, PD spice forms can be put in the following order in terms of their decreasing inhibitory effect towards fungal strains : *Pcit* : *Sa*> *Ccb*> *Ccm*; *Pexp* : *Ccb*> *Sa*> *Ccm*.

Table 2: Effect of different concentrations of PD spices on growth of fungi

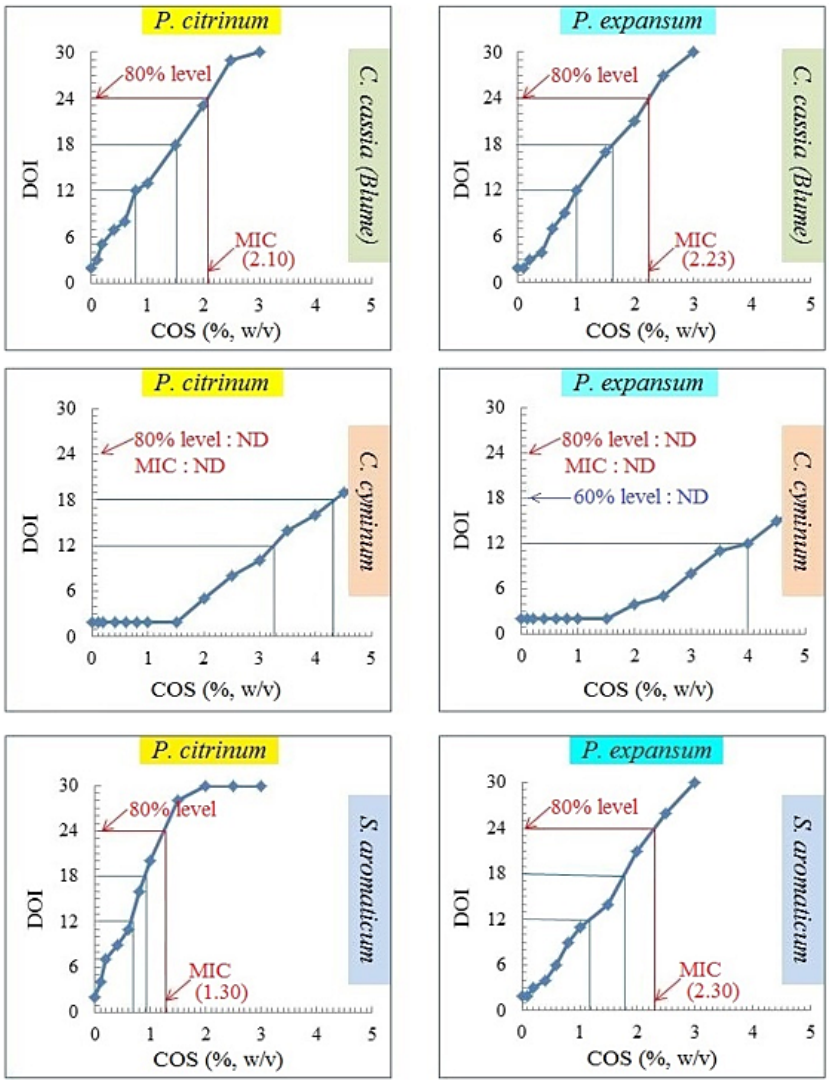
Spice Conc. (%,w/v)	Days of Inhibition							
	As		*Ccb*		*Ccm*		*Sa*	
	Pcit	*Pexp*	*Pcit*	*Pexp*	*Pcit*	*Pexp*	*Pcit*	*Pexp*
0.0	2	2	2	2	2	2	2	2
0.1	2	2	3	2	2	2	4	2
0.2	2	2	5	3	2	2	7	3
0.4	2	2	7	4	2	2	9	4
0.6	2	2	8	7	2	2	11	6
0.8	2	2	12	9	2	2	16	9
1.0	2	2	14	12	2	2	20	11
1.5	2	2	18	17	2	2	28	14
2.0	2	2	23	21	5	4	>30	21
2.5	2	2	29	27	8	5	>30	26
3.0	2	2	>30	>30	10	8	>30	>30
3.5	2	2	>30	>30	14	11	>30	>30
4.0	2	2	>30	>30	16	12	>30	>30
4.5	4	2	>30	>30	19	15	>30	>30
5.0	5	2	>30	>30	21	17	>30	>30

As: Allium sativum, Ccb: Cinnamomum cassia (Blume), Ccm: Cuminum cyminum
Sa: Syzygium aromaticum, Pcit: Penicillium citrinum, Pexp: Penicillium expansum

Table 3 : Levels of inhibition generated by PD spices

Levels of Inhibiton	Spice concentration (%, w/v)					
	Ccb		*Ccm*		*Sa*	
	Pcit	Pexp	Pcit	Pexp	Pcit	Pexp
40%	0.80	1.00	3.28	4.00	0.70	1.20
60%	1.50	1.60	4.30	NOB	0.90	1.60
80%	2.10	2.23	NOB	NOB	1.30	2.30

Ccb*: Cinnamomum cassia (Blume),* Ccm*: Cuminum cyminum,* Sa*: Syzygium aromaticum,*
Pcit: *Penicillium citrinum,* Pexp: *Penicillium expansum,* NOB: *Not observed*



COS: Concentration of spice, MIC: Minimum inhibitory concentration

DOI: Days of Inhibition, ND: Not Detected

Fig. 1: Levels of inhibition generated by PD spices

Inhibitory Activities of Aes and EOs of Spices

Results (Table 4) of impregnated paper disc method show that petriplates poured with AEs of test spices did not show any growth inhibitory zone towards *Pcit* and *Pexp*. *Sa*me response of test fungi was observed towards EOs of Cl, Tf-g and Zo, and also with the control set of petriplates incorporated with DMSO. Contrastingly, EOs of *As*, Bj, *Ccb*, *Ccm*, *Os*, Mp and *Sa* displayed distinct zones of inhibition (mm) of varying diameters towards both the fungi under observation. It is obvious from the data that *Pcit* gave wider growth inhibitory zones as compared to *Pexp* towards all the spice EOs. Based on the measured diameters of inhibitory zones, the following ranking of spice EOs in their descending order can be made towards fungal strains :

Pcit : Ccm> Sa> Ccb> Bj> As> Mp> Os ; Pexp : Sa> Bj> As= Ccm> Ccb> Mp> Os.

MICs of EOs (which exhibited inhibitory zones during impregnated paper disc method), were determined using broth dilution as*say*, and are enlisted in

Table 5. Data revealed that MIC values of EOs towards *Pcit* ranged from 3.90 µL/mL- 62.50 µL/mL, and towards *Pexp*, MIC values varied from 7.81 µL/mL- 62.50 µL/mL. On the basis of MIC values obtained, effectivity of spice EOs in descending order towards fungi can be put as :
Pcit : Ccb= Ccm= Sa> As= Bj= Mp> Os; Pexp : Ccb> As= Bj= Ccm= Sa> Mp> Os.

Table 4: Inhibitory activities of AEs and EOs

Spices	Zones of Inhibition (mm)					
	DMSO (5µL)		EO (5µL)		AE (10µL)	
	Pcit	***Pexp***	***Pcit***	***Pexp***	***Pcit***	***Pexp***
As	NOB	NOB	27.10±0.56	24.00±0.21	NOB	NOB
Bj	NOB	NOB	40.50±0.09	32.00±0.12	NOB	NOB
Ccb	NOB	NOB	41.50±0.16	19.50±0.29	NOB	NOB
Ccm	NOB	NOB	42.10±0.51	24.10±1.00	NOB	NOB
Cl	NOB	NOB	NOB	NOB	NOB	NOB
Mp	NOB	NOB	12.50±0.33	12.00±0.38	NOB	NOB
Os	NOB	NOB	10.30±0.57	10.10±0.12	NOB	NOB
Sa	NOB	NOB	42.00±0.67	34.00±0.50	NOB	NOB
Tf-g	NOB	NOB	NOB	NOB	NOB	NOB
Zo	NOB	NOB	NOB	NOB	NOB	NOB

As: Allium sativum, Bj: Brassica juncea, Ccb: Cinnamomum cassia (Blume),
Ccm: Cuminum cyminum, Cl: Curcuma longa, Mp: Mentha piperita,
Os: Ocimum sanctum, Sa: Syzygium aromaticum, Tf: Trigonella foenum-graecum,
Zo: Zingiber officinale, Pcit: Penicillium citrinum, Pexp: Penicillium expansum,
NOB: Not Observed, DMSO: Dimethylsulphoxide.

Table 5: Minimum inhibitory concentrations (MICs) of EOs

Spices	MICs			
	DMSO		EOs	
	Pcit	***Pexp***	***Pcit***	***Pexp***
As	NOB	NOB	7.81	15.62
Bj	NOB	NOB	7.81	15.62
Ccb	NOB	NOB	3.90	7.81
Ccm	NOB	NOB	3.90	15.62
Mp	NOB	NOB	15.62	62.50
Os	NOB	NOB	31.25	62.50
Sa	NOB	NOB	3.90	15.62

As: Allium sativum, Bj: Brassica juncea, Ccb: Cinnamomum cassia (Blume),
Ccm: Cuminum cyminum, Mp: Mentha piperita, Os: Ocimum sanctum,
Sa: Syzygium aromaticum, Pcit: Penicillium citrinum, Pexp: Penicillium expansum,
NOB: Not Observed, DMSO: Dimethylsulphoxide.

Discussion

Spices are well known for their flavouring and therapeutic properties. Mainstream scientific community readily accepts spices as antimicrobial agents. Present in vitro trials clearly suggest that spice forms under investigation showed marked variability in inhibiting phytopathogenic *Pcit* and *Pexp*. As per our observations, EOs of spices arrested the fungal growth m*os*t potentially, followed by PD spices, whereas AEs were found totally ineffective. Our findings are in consistency with the results of Hetta *et al.*, th*os*e elucidated that EOs and PD forms of some spice plants (black cumin, cinnamon, clove, cumin and marjoram), have antibacterial activities against *Bacillus* spp. isolated from raw and processed meat, to which they further added that inhibitory potential of EOs was stronger than the PD forms of the *sa*me plant.[26]

Furthermore, results of our study revealed that amongst PD forms of spices, *Ccb* and *Sa*, arrested the growth of *Pcit* and *Pexp*, m*os*t significantly followed by *Ccm* and *As*, while Bj, Cl, Mp, *Os*, *Tf-g* and *Zo*, proved ineffectual. This variation in the antifungal activities shown by PD spices may be attributed to their volatile EOs. Rio *et al.* and many others have po*s*tulated that functional properties of spices reside in their volatile EOs, which in turn consist of a myriad of phytochemicals, viz., alcohols (farnesol, menthol), aldehydes (cinnamic aldehyde, cuminic aldehyde), polyphenols (eugenol, resveratrol), terpenoids (thymol) and thiols (allicin and allylisothiocyanate), preferably known as bioactive components.[27,28,29] Aforestated components are basically responsible for the antimicrobial activities of spices, either by causing degradation of cell wall/ cytoplasmic membrane, leakage of cellular components or by affecting their genetic material, protein synthesis, electron transport system, nutrient uptake, enzymatic activities and energy production inside the cell, adversely.[29,30,31]

Therefore, low effectivity of *As* and non effectivity of B*j, Cl, Mp, Os, Tf-g* and *Zo,* in their PD forms in current study, may be either due to their less amount of EOs or due to the substantial lo*s*s of volatile antimicrobial components during the process of drying and grinding. It is quite evident from our results, wherein EOs of *Bj, Mp* and O*s* exhibited growth inhibitory effects, while PD forms of referred spices did not show any antifungal activity. Moreover, greater antifungal potential of *Sa*, *Ccb* and *Ccm* may be attributed to their high amount of EOs and chemical comp*os*ition of EOs. Past researches have demonstrated that *Sa* comprises 15-20% EO, which is primarily eugenol (80-85%).[32] Likewise, *Ccb* also consists of considerable amount of EO that ranges from 0.9-4.0% with major constituent being cinnamic aldehyde,[33] and *Ccm* contains 2.5-4.5% of EO, and is mainly comp*os*ed of cuminic aldehyde.[34] Though, it deserves special attention that amount of EO in *Ccb* is far less as compared to *Sa*, but antifungal activities shown by PD forms of these 2 spices towards *Pcit* and *Pexp*, were cl*os*e to each other as per our observations. Similarly, *Ccm* has more amount of EO as compared to *Ccb*, but in terms of generation of days of inhibition, PD form of former proved less effective than the later. This discrepancy in results, thus can be attributed to different chemical structures of major bioactive components of EOs of spices and their specific mode of action. Other related factors responsible for greater antifungal potentials of PD*Ccb* and PD*Sa* may include the pH, volatility, molecular weight and diffusion of antimicrobial components in growth medium along with type of microorganism implicated in the study. However, exact mechanisms of action of these bioactive components towards *Pcit* and *Pexp* at molecular levels are not yet understood, and would remain a line of future research.

Similar reasons can be given for differential antifungal activities shown by EOs of spices towards *Pcit* and *Pexp*, during broth dilution as*sa*y and impregnated paper disc method, where EOs of Mp and O*s* were less potent in inhibiting test fungi as compared to EOs of *As, Bj*, *Ccb*, *Ccm* and *Sa*, towards both the test fungi. Our results were in agreement with the findings of De-Montijo-Prieto *et al.*, wherein they reported antimicrobial activities of 13 EOs from different herbs, spices, fruits and vegetables towards some common food borne pathogens.[18]

The altogether inert nature of 10 AEs in current trials could be due to the insolubility of hydrophobic antimicrobial components of EOs in water. It is suggested that the hydrophobicity of the EOs and their bioactive components is the detrimental factor which makes them to get accumulated in the lipid bilayer of the cell membrane and mitochondria,

rendering them more permeable to external agents Gonelimali *et al.* also found that aqueous extracts of *Hibiscus sabdariffa* and *Syzygium aromaticum* did not show antimicrobial activity against *Candida albicans.*[19]

It was also notable that inhibitory effect of EOs and PD forms was more pronounced against *Pcit* as compared to *Pexp*. Though, reasons for greater susceptibility of *Pcit* need further elucidation.

Conclusion

Concludingly, *Ccb*, *Ccm* and *Sa*, in their two test forms, i.e., EOs and PD forms, inhibited *Pcit* and remarkably, and hence, may be considered as 'potent inhibitors'. Conversely, *Cl, Tf-g* and Zo did not show any growth inhibitory activity, in their any test form, and may be categorized as 'non inhibitors'. Hence, *PD* forms of *Ccb*, *Ccm* and *Sa*, being cheap and *sa*fe, may find a place as 'natural antimicrobials' along with EOs of *As, Bj, Ccb, Ccm, Mp Os* and *Sa*, to retard the growth of *Pcit* and *Pexp* in order to extend the shelf life of fruits and processed food products. This study would help to reduce p*os*t harvest l*os*s of valuable agricultural commodities and meet the ever increasing consumers' demand of wholesome food which is natural and free from harmful chemical additives.

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Conflict of Interest

Authors have no conflict of interest in this study.

References

1. Shaikh N.U., Chavan A.M. Isolation and characterization of *penicillium* sps. from citrus. *Int J cur res.* 2017; 9: 53465-53466.
2. N*o*sheen A., Anjum T., Jabeen R. Isolation and identification of storage fungi from citrus *sa*mpled from major growing areas of Punjab, Pakistan. *Int J Agr Biol.* 2013; 15: 1283–1288.
3. *Sa*leh I., Al-Thani R. Fungal food spoilage of supermarkets' displayed fruits. *Vet World.* 2019; 12: 1877-1883. DOI:10.14202/vetworld.2019.1877-1883
4. Kim W.K., *Sa*ng H.K., Woo S.K., Park M.S., Paul N.C., Hun Yu S. Six species of *Penicillium* associated with blue mold of grape. Mycobiol. 2007; 35: 180-185. DOI:10.4489/MYCO.2007.35.4.180
5. Coutinho T.C., Ferreira M.C., R*osa* L.H., de Oliveira A.M., de Oliveira E.N. Júnior. *Penicillium citrinum* and *Penicillium* mallochii: New phytopathogens of orange fruit and their control using chit*osa*n. Carb Poly. 2020; 234: 01-10. DOI: 10.1016/j.carbpol.2020.115918
6. Estrada R.R.G., de Jesus Ascencio Valle F., *Sa*nchez J.A.R., *Sa*ntoy M.C. Use of a marine yeast as a biocontrol agent of the novel pathogen *Penicillium citrinum* on Persian lime. Emi J Food Agr. 2017; 29: 114-122. DOI: 10.9755/ejfa.2016-09-1273
7. Frimpong G.K., Adekunle A.A., Ogundipe O.T., Solanki M.K., *Sa*dhasivam S., Sionov E. Identification and toxigenic potential of fungi isolated from *Capsicum* Peppers. *Microorganisms*. 2019; 7 : 01-10. DOI: 10.3390/microorganisms7090303
8. Kamle M., Mahato D.K., Gupta A., Pandhi S., Sharma N., Sharma B. Citrinin mycotoxin contamination in food and feed : Impact on agriculture, human health, and detection and management strategies. *Toxins.* 2022; 14: 01-25. DOI: 10.3390/toxins14020085
9. Batta Y.A. Quantitative p*os*tharvest contamination and transmission of *Penicillium expansum* (Link) conidia to nectarine and pear fruit by *Drosophila maelanogaster (Meig.) adults. Postharv Bio Technol.* 2006; 40: 190-196. DOI:10.1016/j.p*os*tharvbio.2006.01.006.
10. Molinu M.G., Pani G., Venditti T., Dore A., Ladu G., D'Hallewin G. Alternative methods

to control p*os*tharvest decay caused by *Penicillium expansum* in plants *(Prunus domestica L.). Commun Agr App Biol Sci.* 2012; 77: 509-514.

11. Ivana V., Duduk N., Vasic M., Nikolic M. Identification of *Penicillium expansum* causing p*os*tharvest blue mold decay of apple fruit. *Pestic Phytomed.* 2014; 29: 257–266. DOI: 10.2298/PIF1404257V
12. Kim J., Lee W., Cheong S., Choi J., Ryu J., Choi Y. Identification and characteristics of *Penicillium* spp. isolated from p*os*tharvest decay of pear. Res Plant Dis. 2002; 8: 107-112.
13. *Sa*leh I., Goktepe I. Health risk assessment of Patulin intake through apples and apple-based foods sold in Qatar. *Helion.* 2019; 5: 01-07. DOI: 10.1016/j.heliyon2019.e02754
14. Jimenez M., Maldonado M.I., Rodriguez E.M., Hernandez-Ramirez A., *Sa*ggioro E., Carra I., *et al.* Supported TiO2 solar photocatalysis at semi-pilot scale : degradation of pesticides found in citrus processing industry waste water, reactivity and influence of photogenerated species. *J Chem Tech Biotech.* 2015; 90: 149–157. DOI: 10.1002/jctb.4299
15. Talibi I., Boubaker H., Boudyach E., Ben Aoumar A. Alternative methods for the control of p*os*tharvest citrus diseases. *J App Micro.* 2014; 117: 01–17. DOI: 10.1111/jam.12495
16. Fischer I., Lourenco S., Amorim L. P*os*tharvest diseases in citrus and characterization of the fungal population in *Sa*o Paulo's whole*sa*le market. *Trop Plant Path.* 2008; 33: 219–226.
17. Thomas L., *Sa*nil P.C. Competitiveness in spice export trade from India : A review. *J Spices Arom Crops*. 2019; 28: 01-19. DOI:10.25081/j*osa*c.2019.v28.i1.5738
18. De-Montijo-Prieto S., Razola-Díaz M.C., Gómez-Caravaca A. M., Guerra-Hernandez EJ, Jiménez-Valera M, Garcia-Villanova B, *et al.* Essential oils from fruit and vegetables, aromatic herbs, and spices: compo*s*ition, antioxidant, and antimicrobial activities. *Biology*. 2021; 10: 01-21. DOI: 10.3390/biology10111091
19. Gonelimali F.D., Lin J., Miao W., Xuan J., Charles F., Chen M. and Hatab S.R. Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Front Microb.* 2018; 9: 01-09. DOI : 10.3389/fmicb.2018.01639
20. Bhatwalkar S.B., Mondal R., Krishna S.B.N., Adam J.K., Govender P., Anupam R. Antibacterial properties of organo*s*ulfur compounds of Garlic *(Allium sativum). Front Microb.* 2021; 12: 01-20. DOI: 10.3389/fmicb.2021.613077
21. Vasconcel*os* N.G., Croda J., Simionatto S. Antibacterial mechanisms of cinnamon and its constituents: A review. *Microb. Path.* 2018; 120: 198-203. DOI:10.1016/micpath2018.04.036
22. Jayapal V. Antimicrobial activity of eugenol against human pathogenic bacteria by minimal inhibitory concentration, minimal bactericidal concentration and disc-diffusion methods. *Int J Pharm Sci Res.* 2021; 12: 330-335. DOI: 10.13040/IJPSR.0975-8232.12(1).330-35
23. Mahmud J., Khan R.A. Characterization of natural antimicrobials in food system. *Adv Microb.* 2018; 8: 894-916. DOI: 10.4236/aim.2018.811060
24. Rajkumar V., Berwal J.S. Inhibitory effect of clove *(Eugenia caryophyllus)* on toxigenic molds. *J Food Sci Tech.* 2003; 40: 416-418.
25. Kim H.O., Park S.W., Park H.D. Inactivation of Escherichia coli 0157:H7 by cinnamic aldehyde purified from Cinnamomum cassia shoot. *J Food Microbiol* 2004; 21: 105-110.
26. Hetta H.F., Meshaal A.K., Algammal A.M., Yahia R., Rabab R., Makharita R.R. In-vitro antimicrobial activity of essential oils and spices powder of some medicinal plants against *Bacillus* species isolated from raw and processed meat. *Inf Drug Res.* 2020;13: 4367–4378.DOI: 10.2147/IDR.S277295
27. Gutierrez-del-Rio I., Fernandez J., Lombo F. Plant nutraceuticals as antimicrobial agents in food preservation: terpenoids, polyphenols and thiols. *Int J of Antimicrob Agents.* 2018; 52:309-315. DOI: 10.1016/j.ijantimicag.2018.04.024
28. Papadochristopoul*os* A., Kerry J.P., Fegan N., Burgess C.M., Duffy G. Natural anti-microbials for enhanced microbial *sa*fety and shelf-life of processed packaged meat. *Foods.* 2021; 10: 01-42. DOI: 10.3390/

foods10071598

29. Chouhan S., Sharma K., Guleria S. Antimicrobial activity of some essential oils-present status and future perspectives. *Medicines.* 2017; 4: 01-21. DOI:10.3390/medicines4030058
30. *Sa*nt D.G., Tupe S.G., Ramana C.V., Deshpande M.V. Fungal cell membrane-promising drug target for antifungal therapy. *J Appl Microbiol.* 2016; 121: 1498-1510.
31. Angane M., Swift S., Huang K., Butts C.A., Quek S.Y. Essential oils and their major components: an updated review on antimicrobial activities, mechanism of action and their potential application in the food industry. *Foods.* 2022; 11: 01-26. DOI: 10.3390/foods11030464
32. Haro-González J.N., Castillo-Herrera G.A., Martínez-Velázquez M., Espin*osa*-Andrews H. Clove essential oil (*Syzygium aromaticum L. Myrtaceae*) : Extraction, chemical compo*s*ition, food applications, and essential bioactivity for human health. *Molecules.* 2021; 26: 01-25. DOI: 10.3390/molecules26216387
33. Chahbi A, Nassik S, Amri H, Douaik A, Maadoudi EHE, Boukharta M, Hadrami EME. Chemical compo*s*ition and antimicrobial activity of the essential oils of two aromatic plants cultivated in Morocco *(Cinnamomum cassia and Origanum compactum). J Chem.* 2020; 2020: 01-10. DOI: 10.1155/2020/1628710
34. Dubey P.N., *Sa*xena S.N., Lal G., Kant K., Mishra B.K., Agarwal D., John S. and Sharma L.K. Essential oil constituents and un*sa*turated fatty acids in Indian *Cuminum cyminum* L. seed oil under varying agro climatic environments. *Int J seed spices*. 2018; 8: 7-14.