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Review paper

Spice aroma to prevent fungal food spoilage!

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Abstract

Spices are commonly used to impart their unique aroma and taste in the food preparations. Their extracts have proved antimicrobial by directly acting on them. The main objective of this study is to determine the antibacterial and antifungal effects of spices aroma from the distance of 2 cm and not their extracts. Aroma of 8 different Indian spices have assessed against Bacillus, Aspergillus and Penicillium spp. The spices used for this study were bought fresh from the local markets of Mangalore. Collected spices were clove, cumin, cinnamon, star anise, asafoetida, nutmeg and mace, pepper, carom seeds. Aroma of surface sterilized whole spices individually and in a mixture of eight was exposed to the microbes in the NA and SDA plates.

The result states that cumin and clove were more effective against Bacillus than the other spices; cinnamon was more effective against Aspergillus while cinnamon and carom seeds were more effective against Penicillium. Mixture of spices was more antimicrobial than individual spices but less antibacterial and more antifungal in action. In addition to the various uses of spice aroma as taste maker in food preparation, as room fresher and as pest repellent in museum, aroma of these spices mix can be used to preserve the cereals, pulses and grains thereby preventing fungal spoilage.

Keywords- Spice; aroma; antibacterial; antifungal; food spoilage

Introduction

Spices are an important part of Indian cooking and find utility in all the cultures of the world. There are more than thirty spices and herbs of global economic and culinary importance. Spices can be buds (clove), bark (cinnamon), root (ginger), berries (grains of pepper), seeds (cumin), leaf (bay leaf), or even stigma of the flower (saffron).(1) The most important spice crops are black pepper, capsicums nutmeg cinnamon ginger turmeric cloves, coriander, thyme mint mustard, sesame seeds. (2, 3)

Spices have been used since ancient times for flavour, colour, aroma, preservation of food or beverages and traditional medicines due to their unique aroma.. This aroma is due to the sufficiently volatile compound present in it including essential oils. Spices are also used as disinfectants, insecticides, in shelves and museum to keep away the pests, as room freshener, in garnishing and to control bad breath. They also have lots of health benefits due to the secondary metabolites, polyphenols, terpenoids, alkaloids, lectins,

polypeptides, and polyacetylenes are known to be antimicrobial agents (4, 5).

In the present study, an attempt has been made to investigate the antimicrobial effects of aroma of eight Indian spices individually and in a mixture. Our study deals with the investigation of 8 spices, against a Gram-positive Bacillus, Aspergillus and Penicillium. Earlier studies report the antimicrobial effect of Spice by using its extract or essential oils directly acting on the microbes. Whereas in this study we have used the aroma of spices on the bacteria and fungi without being in direct contact with microbes. This study is the first of its kind. As this method is commonly used as insect and pest repellent in museums to protect the handwritten scriptures and ancient documents. Similar concept has been tried to verify in the laboratory by testing the aroma of spices on common food spoilage microbes like Bacillus, Aspergillus and Penicillium spp.

Methodology:

Collection of spices:

The spices used for this study were bought fresh from the authentic Ayurveda shop of Mangalore Market. The collected spices were clove (*Syzygium aromaticum*), cumin (*Cuminum cyminum*), cinnamon (*Cinnamomum zeylanicum*), star anise (*Illicium verum)*, asafoetida (*Ferula asafoetida*), nutmeg and mace (*Myristica fragrans*), pepper (*Piper nigrum*), carom seeds (*Trachyspermum ammi*).5gram of each spice was used for individual aroma effect on microbes and even for the mixture same quantity was weighed and mixed together to check the antimicrobial effect.

The test microorganisms. These were *Bacillus subtilis, Aspergillus niger* and *Penicillium Chysogenum*. For bacterial study, Nutrient Agar and for fungal study Sabouraud Dextrose Agar was used. All 8 spices were weighed 5 grams and taken for study to test the antimicrobial effect of its aroma separately and collectively as mixture of all 8 spices. All the spices were surface sterilized under U V light for 15 min and taken in the lid of sterile petriplates and allowed the aroma to get saturated in the petriplates. The turbidity of the 18 hour old bacterial culture was adjusted to 0.5 McFarland standards (equivalent to 1.5 × 108 CFU/ml). and spread on the sterile nutrient agar and inverted the plate base on the lid containing spices.(6) There was a gap of 2 cm between the Bacillus culture inoculated on the nutrient agar and the spice surface present in the lid.

Thus all the 8 spices were kept separately in the 8 plate exposed to inoculated Bacillus culture. One plate without spice was considered as control plate. The plates were incubated for 24 hours at 37^{o}C.and growth was observed next day in all the plates. In the second set, all the 8 spices, each weighted 5 gms, and sterilized under U V light and mixed togather. This mixture of 8 spices was kept in a petri plate lid. Remaining procedure was same as like individual spices..

For fungi, 24 hour old culture was taken and culture suspension was adjusted to 1.0 McFarland standard (equivalent to 1.5 × 108 CFU/ml) and similar procedure was repeated by exposing all the spices separately and in a mixture of 8 spices to the Aspergillus and Penicillium culture inoculated on the SDA in different experiments. The plates were incubated for 3 days at room temperature. One SDA plate without spices was kept as control. Growth was observed after 3 days. All the eight spices used in the study are given in the figure 1.

Result

The bacterial and fungal growth in response to individual and mixture of spices is given in the Figure 2, 3, 4 and 5 and Table 1,2, 3,4 and 5, after 24 hours and 3 days respectively. Overall result shows that mixture of spices was more effective than individual spices but less antibacterial and more antifungal permitting slow growth with or without spore formation. In the individual spice study, only clove and cumin seeds were slightly effective as antibacillus. Whereas Cinnamon and clove proved to be anti Aspergillus than other spices. For *Penicillium chrysogenum*, Cinnamon clove and star anise proved antifungal in nature.

Discussion

Antimicrobial effect of spices has been studied till now by various researchers using various extracts and by direct contact of spices on microbes. But our study had assessed the effect of volatility of spice aroma on *Bacillus subtilis* and common fungi, Aspergillus and Penicillum spp without direct contact. The aroma of mixture of spices exhibited moderate growth of Bacillus. It also showed slow growth of Aspergillus and Penicillium spp. Thus Aroma of mixture of spices was more effective on Penicillium than Aspergillus spp.

Aroma effect on Bacillus

The aroma of cumin and clove showed moderate antibacterial effect on Bacillus. Our study shows similar results to that of Pavithra .This effect may be due to its strong, pungent and spicy odour and taste (7). Aroma of nutmeg showed no antibacterial effect on Bacillus. In decreasing order the antibacterial effect of spices on Bacillus was – clove, cumin, pepper, cinnamon, carom seeds, star anise, asafoetida and nutmeg. Our findings do not match with results reported earlier by others as nutmeg is effective antimicrobial only on direct contact effect. The Carvacol and thymol present in carom seeds are reported as either bactericidal or bacteriostatic. (8) Our finding do not show such effect.

Similarly the findings of A. Augustine and G. Sreeraj states the anti-bacterial effect of asafoetida whereas our findings do not correlate with that of (9) Even the results of star anise too do not match with the findings of Singh and Nik nor Where they have used aqueous and crude extracts of essential oils and observed the effect with direct contact with microbes to control the food contaminating bacteria. It is also reported that an active compound anethole of star anise has shown higher antimicrobial property against bacteria, yeast, fungal strains (10, 11) .The result varies due to the method used to study. Some of the spices aromas used in our study were showing less or no antibacterial and antifungal effect may be due to the aroma which is not effective like the extract taken from the spices and assessed based on the direct contact methods like well diffusion method, disc diffusion, minimum inhibition concentration and Minimum bactericidal concentration (MIC, MBC) method used by other reporters.

Antifungal effect of aroma of individual spice-

Aroma effect on Aspergillus- Aroma of Cinnamon showed more antifungal effect on *Aspergillus niger* on 3 rd. day with slow mycelial growth. This can be subjected to the main compounds in cinnamon polyphenols and volatile phenols. (12) The cumin aroma showed least antifungal effect on *Aspergillus niger* on 3 rd. day with slow growth of mycelia.The decreasing order of antifungal effect of individual spice aroma on *Aspergillus niger* on 3 rd. day was – cinnamon, clove, pepper, carom seeds, star anise, asafoetida, nutmeg and cumin. On 4th day all the plates with individual spices showed growth of mycelia. The effect of pepper can be attributed to its phenolic compounds and

essential oil composed primarily of monoterpenes and sesquiterpenes inhibiting the growth of microorganisms (13). In our case antifungal effect may be due to the volatile essential oil of pepper.

Nutmeg has shown no antifungal effect in our observation. Whereas Dzotam *et.al.*, reports that Nutmeg Shows antimicrobial activity against a wide range of spoilage and pathogenic bacteria and aroma from *Myristica fragrans* seeds has growth inhibition capability of bacterial spores and can be used as food preservative. (14) This result is based on its methanol extract used to assess the antifungal activity by MIC method.

Cinnamon aroma followed by clove and star anise showed very slow growth of mycelia. Cumin and asafoetida aroma did not show any antifungal effect on *Penicillium chrysogenum* on day 4. Thus cinnamon was found more antifungal against Aspergillus *niger* compared to other spices.

Aroma effect on Penicillium-Aroma of clove, cinnamon and carom seeds showed more antifungal effect on Penicillium on 3 rd. day with no growth. The aroma of cumin and nutmeg showed least effect on Aspergillus. The decreasing order of antifungal effect of individual spices on Penicillium on 3 rd. day was – cinnamon and carom seeds, clove, star anise, pepper, asafoetida, cumin and nutmeg. Therefore, cinnamon and carom seeds aroma proved to be more antifungal against Penicillium than the other spices.

Thus various active compound present in the spices gets concentrated when extracted along with its essential oils and it is more effective when acts on microbes directly. On the contrary the aroma of spices is mainly due to the volatile compounds present in them. This study has helped to assess the effect of these volatile compounds on Bacillus, Aspergillus and Penicillum spp from the distance of 2 cm in closed environment. Based on these results, the spices studied here can be used effectively as more of antifungal agents than a for antibacterial purpose. As only aroma is imparting the effect, the whole spice can be reused multiple times. Further scope of the study resides in experimenting with all food spoilage microorganisms especially fungi exposing to the aroma of multiple combination of spices.

Conclusion

Our study reports that these eight spices work better as antimicrobial when in the mixture than individual. They showed less antibacterial and more antifungal activity. To prevent food spoilage, Spices like Clove, cinnamon, cumin seeds and star anise aroma can be used as effective antifungal agents. This outcome can be put in to practice to preserve cereals, pulses and grains to prevent fungal food spoilage.

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Conflict of interest- Authors declares no conflict of interest.

Compliance with ethical standards- Authors declare that this study does not involve animal or human participation.

Authors Contribution-Dr Bharathi Prakash designed the study and prepared the manuscripts .Other authors were involved in the project research work.

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Figures

Figure 1- The spices used for the study.

Figure-2).Control plate (dense growth) ,Test plate (moderate growth).with mixture of spices with *Bacillus Subtils*

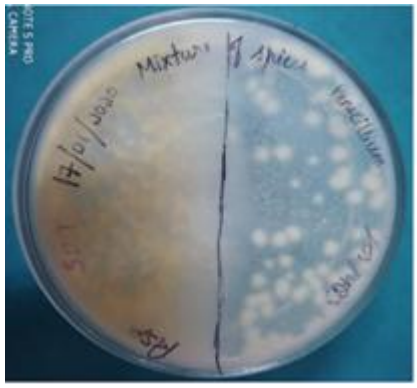

Figure-3): Control plate for Aspergillus and Penicillium.

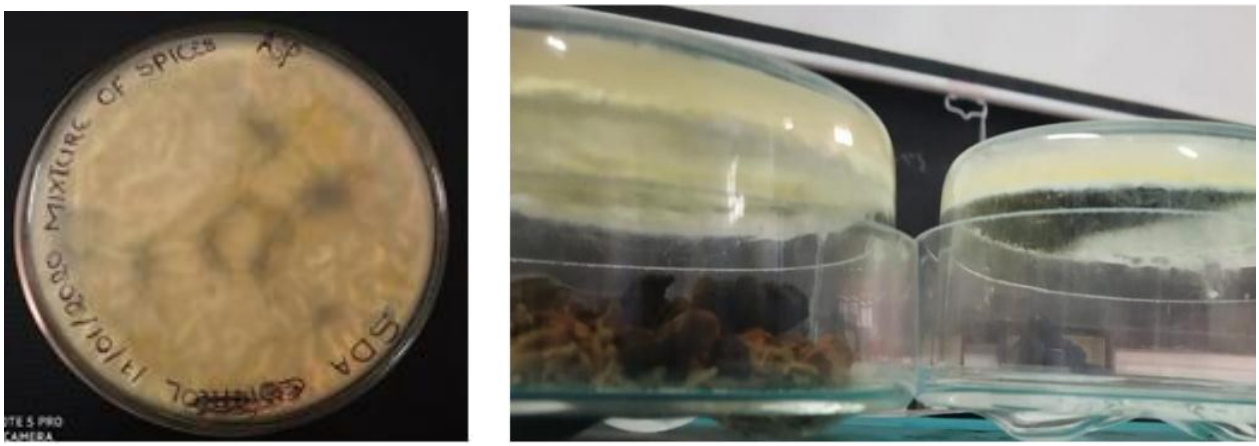

Figure-4): Antifungal effect of mixture of spices against Aspergillus in test plate without spores and control plate with spores

Figure-5): Antifungal effect of mixture of spices against Penicillium in test and control plate. without spores

Tables

Table 1: Antibacterial effect of mixture of spices against *Bacillus subtilis*

Control plate	Test plate
Dense growth	Moderate growth

Table 2: Antifungal effect of mixture of spices against Aspergillus and Penicillium spp.

DAY	Aspergillus		Penicillium	
	Control plate	Test plate	Control plate	Test plate
3	Spore formation	Slow mycelial growth	Mycelial growth	No growth
4	Spore formation	Slow mycelial growth	Mycelial growth	No growth
5	Spore formation	Slow mycelial growth	Mycelial growth	No growth
6	Spore formation	Slow growth of spores	Spore formation	Slow Mycelial growth

Table 3: Antibacterial effect of individual spices against *Bacillus Subtilis.in* 24 hr of incubation.

Spices (5g)	Control plate	Test plate
Clove	++++++	+ + +
Cinnamon	++++++	+++++
Cumin	++++++	+ + +
Pepper	++++++	++++
Nutmeg and mace	++++++	++++++
Carom seeds	++++++	+++++
Asafoetida	++++++	+++++
Star anise	++++++	+++++

Note-+ +scanty growth, + ++ =slow growth, ++++=moderate growth ++++++=heavy growth with spores

Table 4: Antifungal effect of individual spice against Aspergillus.

SPICES (5g)	CONTROL PLATE (spore formation)	TEST PLATE DAY 3	TEST PLATE DAY 4 (spore formation)
Clove	++++++	+ + +	+ + +
Cinnamon	++++++	+	+ +
Cumin	++++++	+ + + + + (spore formation)	++++++
Pepper	++++++	++++	+++++
Nutmeg and mace	++++++	++++	+++++
Carom seeds	++++++	++++	+++++
Asafoetida	++++++	++++	++++++
Star anise	++++++	++++	++++

Note -+ and + += scanty growth, + ++ =slow growth,++++++=heavy growth

Table 5: Antifungal effect of individual apices against Penicillium .

SPICES (5g)	CONTROL PLATE (spore formation)	TEST PLATE DAY 3	TEST PLATE DAY 4 (spore formation)
Clove	++++++	-----	+
Cinnamon	++++++	-----	-----
Cumin	++++++	+++++	+++++
Pepper	++++++	++++	++++
Nutmeg and mace	++++++	+++++	+++++
Carom seeds	++++++	-----	-----
Asafoetida	++++++	++++	++++
Star anise	++++++	+ + +	+ + +

Note -+ and + += scanty growth, + ++ =slow growth,++++++=heavy growth