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Evaluation of antifungal activities of some Indian dietary spices against pathogenic *Rhizopus arrhizus* and *Rhizopus stolonifer*

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Abstract

Rhizopus arrhizus and *Rhizopus stolonifer* are pathogenic fungi responsible for causing 'soft rot' disease in fruits and vegetables, as well as life-threatening 'mucormycosis' in animals and humans, especially immunocompromised hosts. The use of synthetic fungicides and chemical preservatives to inhibit microbes is being challenged due to their toxic effects on human health and the environment. This has prompted researchers to investigate novel antimicrobials that are natural and safe. Current study aimed to evaluate the antifungal activities of essential oils (EOs), powdered (PWD) forms, and water extracts (WEs) of ten Indian dietary spices (*Allium sativum (Als)*, *Brassica nigra (Brn)*, *Cinnamomum zeylanicum (Ciz)*, *Cuminum cyminum (Cuc)*, *Curcuma longa (Cul)*, *Mentha piperita (Mep)*, *Ocimum sanctum (Ocs)*, *Syzygium aromaticum (Sya), Trigonella foenum-graecum (Trf)*, and *Zingiber officinale (Zio)*) against *R. arrhizus* (NCIM877) and *R. stolonifer* (NCIM1139). Spice agar method was used for investigating antifungal potential of PWD spices. Impregnated paper disc method and broth dilution technique were followed for screening the antifungal activities of EOs and WEs. Results revealed that EOs of six spices (*Als*, *Brn, Ciz, Cuc, Mep, Sya*), and PWD forms of four spices (*Brn, Ciz, Cuc, Sya*) inhibited the growth of both fungi in culture media, effectively. WEs of all ten spices remained ineffective in arresting the growth of tested fungal strains. *R. stolonifer* was found more sensitive compared to *R. arrhizus* towards tested substances. Based on these findings, further *in vivo* studies on PWD forms of *Ccb*, *Ccm,* and *Sya* and EOs of *Als, Brn, Ccb, Ccm, Mep, Ocs* and *Sya* may establish spices as 'botanical antimicrobials' to prolong the shelf life of valuable horticultural crops by arresting the growth of *R. arrhizus* and *R. stolonifer*.

Key words: Antifungal, mucormycosis, *Rhizopus arrhizus*, *Rhizopus stolonifer*, soft rot, spices.

Introduction

Rhizopus arrhizus (formerly *Rhizopus oryzae*) and *Rhizopus stolonifer* (syn. *Rhizopus nigricans*) are saprophytic zygomycete fungi of the family *Rhizopodaceae* and order Mucorales, dwelling in diverse habitats including air, soil, water, animal excrement, and decaying vegetation, worldwide (Spatafora *et al.,* 2016; Gryganskyi *et al.,* 2018). The mentioned fungal species of the genus *Rhizopus* act as opportunistic pathogens causing 'soft rot' disease on more than 200 fruit and vegetable crops at pre-harvest and post-harvest stages, and are often characterized by their fast cottony growth and capacity to produce large quantities of spores (Baggio *et al.,* 2016). *R. arrhizus* grows as loose filaments carrying brownish to greyish-black spores (Trabelsi *et al.,* 2020), whereas *R. stolonifer* grows as entangled filaments/tufts with typically black spores (Bijoy and Ahlawat, 2023).

In agricultural commodities, *R. arrhizus* and *R. stolonifer* have been successfully isolated from fresh and stored apricot, apples, bananas, cassava tubers, eggplant, fig, grapes, guava, jackfruit, nectarine, papaya, peach, pear, plum, potato tubers, stone fruit, strawberry, sugar beet, sweet potato, sweet cherry, and tomato (Sweanya *et al.,* 2020; Hartanti *et al.,* 2020; Haque *et al.,* 2023). These two fungi penetrate the soft tissues of the produce, leading to watery leakage and rendering them completely inedible (Zhang *et al.,* 2023). This results in substantial economic losses for farmers and distributors.

R. stolonifer (common bread mold) is generally observed as the first mold to appear on stale bread (Axel *et al.,* 2017). Referred fungal strain has been found in yogurt, cheese, jellies, syrups, and groundnuts leading to the development of unpleasant flavors and changes in texture, making the affected food products unpalatable and unsafe for consumption (Martin *et al.,* 2021).

Inhalation of spores of *R. arrhizus* and *R. stolonifer* and consuming contaminated foods can cause 'mucormycosis' (black fungus disease) in animals and humans (Dannaoui and Lackner, 2019). This rare but dangerous fungal infection is known for high mortality rates and affects various parts of the body such as blood vessels, brain, eyes, intestine, lungs, sinuses, skin and stomach resulting in headache, blurred vision, bulging or displacement of the eye (proptosis), facial swelling, fever, necrosis and thrombosis (Kaerger *et al.,* 2015). Although people of any age can get infected, those with weakened immune systems such as diabetics, cancer patients, organ transplant recipients, and people with HIV/AIDS are more susceptible to the disease (Skiada *et al.,* 2020). During the COVID-19 pandemic, there were reports of an association between mucormycosis and COVID-19 patients taking immunosuppressive drugs, particularly glucocorticoids (Singh *et al.,* 2021; Sharma and Goel, 2022).

The rapid penetration and colonization of *R. arrhizus* and *R. stolonifer* in a wide array of hosts, as well as the serious threat they pose to the global food supply chain, make it crucial to control them. Synthetic fungicides and chemical preservatives are commonly used for this purpose, but their use is increasingly questioned by consumers due to their negative impact on human health and environment. As a result, scientists are continuously seeking natural alternatives.

Spices and spice products are of plant origin and have been used as culinary adjuncts and therapeutics since ancient times. India is known as the 'spice bowl' of the world with its rich spice heritage and production dominance (Gidwani *et al.,* 2022). Owing to their immunity-boosting properties, the demand for Indian spices has risen even more since the outbreak of COVID-19, all over the world (Gidwani *et al.,* 2022). Moreover, in last few decades, many studies have shown that alcoholic extracts, EOs and bioactive components of spices such as cinnamon, clove, coriander, garlic, fennel, oregano, sage, and thyme have antimicrobial properties against various harmful microbes (Bhatwalkar *et al.,* 2021; De-Montijo-Prieto *et al.,* 2021).

Taking the above aspects into account, EOs, PWD forms, and WEs of ten spices commonly used as 'culinary addendums' in Indian kitchens were investigated for their antifungal activities towards pathogenic *R. arrhizus* and *R. stolonifer*.

Materials and methods

Procurement and preparation of PWD spice samples: Plant parts of *Als* (cloves), *Mep* (leaves) and *Zio* (rhizomes), were procured in their fresh forms, from a local grocery shop. *Ocs* leaves were hand plucked from a home grown plant. Peels of *Als* bulbs and *Zio* 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 *Brn* (seeds), *Ciz* (bark), *Cuc* (seeds), *Cul* (rhizomes), *Sya* (flower buds) and *Trf* (seeds) 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 (PWD) forms with the help of a grinder in the Labouratory and were stored in hermetically sealed jars till their further use.

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 %.

WEs of spices: WEs of spices were prepared by the method described elsewhere (Yin and Cheng, 1998). All the WEs 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.

Fungal strains and growth conditions: Pure cultures of *Rhizopus arrhizus* (NCIM 877) and *Rhizopus stolonifer* (NCIM1139) were procured from National Collection of Industrial Microorganisms (NCIM), India. Potato dextrose agar (PDA) and Potato dextrose broth (Hi Media, India), were used for the cultivation of both the fungi (temperature of incubation: 25^o C; duration of incubation: 48 h-96 h), as per the recommendations of NCIM. Growth of *R. arrhizus* appeared as brownish white mycelium with loose hyphae on PDA, whereas growth of *R. stolonifer* appeared as greyish white mycelium with entangled tuft like hyphae on PDA.

Preparation of fungal inocula: For the preparation of fungal inocula, brownish black spores of *R. arrhizus* and black spores of *R. stolonifer* were harvested from their respective 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 10^7spores/mL.

Screening antifungal activities of PWD spices, EOs and WEs

Spice agar method: For the determination of antifungal activities of PWD spice samples, spice agar method was used (Rajkumar and Berwal, 2003). Freshly prepared fungal inoculum (100 µL) was evenly spread over the surface of solidified PDA petri plates previously supplemented with various concentrations of PWD spices (0.1 - 5.0 (%,w/v)). Seeded petri plates were incubated at 25ºC 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 PWD spice sample), was also conducted, side by side.

From the results of spice agar method, minimum inhibitory concentrations (MICs) of PWD spice samples were derived. For the sake, concentrations of spices (COS (%, w/v)) were plotted on the x-axis and days of inhibition (DOI) were plotted on the y-axis of graph (Fig. 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 sample. MIC was the concentration of PWD spice sample which did not allow the fungi to grow up to 80% level, *i.e.,* 24 days.

Impregnated paper disc method: Antifungal activities of WEs and EOs of spices were screened by using impregnated paper disc method (Kim *et al.,* 2004). 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 assay: The broth dilution assay (Kim *et al.,* 2004) aimed to determine and compare the minimum inhibitory concentrations (MICs) of spice essential oils (EOs) against *R. arrhizus* and *R. stolonifer*. MIC represented the lowest EO concentration that, under specific *in vitro* conditions, inhibited the visible growth of fungal strains within a defined time frame. These MIC values will guide researchers on the fungi's susceptibility/resistance to the tested EOs and aid in selecting suitable EO dosages for *in vivo* applications in studying the shelf-life of raw horticultural products and processed food items. For the determination of MICs of EOs, PDB containing 2000 µL/mL of EOs was serially diluted twofold with PDB to obtain 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, and 0.06 µL/mL. Subsequently, 100 µL of freshly prepared fungal inoculum was added to them. The mixtures thus obtained were incubated at 25ºC for 96 hours. After the stipulated incubation period, 100 µL of the above mixture was spread on the surface of solidified PDA plates. The plates were then incubated for 96 hours at 25 ºC to observe the lowest dilution of EOs (MICs) that did not exhibit any visible growth of fungi.

During impregnated paper disc method and broth dilution assay, dimethylsulphoxide (DMSO) (CDH, India), was used as a negative control agent. All the above experiments were conducted in triplicates.

Statistical analysis: Results of impregnated paper disc method were analyzed using statistical analysis software SPSS version 7.5 and were expressed as the Mean ± SD.

Results

Antifungal activities of PWD spice samples: Results of present investigation (Table 1) revealed that PDA petri dishes supplemented with PWD forms of *Brn, Ciz, Cuc* and *Sya*, at different concentrations (0.1-5.0 (%, w/v)), arrested the growth of *R. arrhizus* NCIM877 (*Ra*) and *R. stolonifer* NCIM1139 (*Rs*). Conversely, *Cul, Mep, Ocs, Trf* and *Zio*, up to their highest concentration level of 5.0%, were found ineffective in inhibiting the growth of both the fungal strains under observation, and their growth in culture media was observed on the 2nd day of incubation as in control set of petri dishes without any PWD spice sample. However, *Als* displayed very weak inhibitory effect only towards *Rs*, and former at 5.0% level, delayed the growth of later by 6 days.

Table 1. Effect of different concentrations of PWD spices on growth of fungi

Spice Conc. (%,w/v)	Days of inhibition									
	Als		*Brn*		*Ciz*		*Cuc*		*Sya*	
	Ra	*Rs*	*Ra*	*Rs*	*Ra*	*Rs*	*Ra*	*Rs*	*Ra*	*Rs*
0.0	2	2	2	2	2	2	2	2	2	2
0.1	2	2	2	2	2	4	2	2	2	3
0.2	2	2	2	2	2	7	2	2	2	5
0.4	2	2	2	2	3	10	2	2	3	7
0.6	2	2	2	2	5	14	2	2	4	9
0.8	2	2	2	2	9	16	2	2	5	14
1.0	2	2	2	4	11	22	2	2	9	19
1.5	2	2	4	5	14	28	2	2	12	23
2.0	2	2	7	7	18	>30	4	4	16	29
2.5	2	2	9	9	21	>30	7	6	21	>30
3.0	2	2	11	14	27	>30	8	9	26	>30
3.5	2	2	12	18	>30	>30	10	12	>30	>30
4.0	2	2	14	23	>30	>30	13	15	>30	>30
4.5	2	3	19	27	>30	>30	14	18	>30	>30
5.0	2	6	24	>30	>30	>30	18	20	>30	>30

Ra: Rhizopus arrhizus, Rs: Rhizopus stolonifer, Als: Allium sativum, Brn: Brassica nigra, Ciz: Cinnamomum zeylanicum, Cuc: Cuminum cyminum, Sya: Syzygium aromaticum

It was observed that number of days of inhibition increased with the increase in the concentrations of PWD spices. And, *Ciz* and *Sya* at 2.0% and 2.5% levels, inhibited the growth of *Rs* for more than 30 days, while visible growth of *Ra* was delayed by 30 days, at 3.5% level of *Ciz* and *Sya*. *Brn* up to its highest concentration level of 5.0% inhibited *Rs* for the desired incubation period of 30 days, while *Ra* was inhibited up to 24 days. *Cuc* at 5.0% level, delayed the visible growth of *Ra* up to 18 days, and that of *Rs* up to 20 days, and was thus unable to produce the required growth inhibitory effect even at its highest stated concentration level.

It was noted that different levels of inhibition were generated by different concentrations of referred spices towards both the fungi under investigation (Fig. 1, Table 2). *Brn, Ciz* and *Sya* produced 40%, 60% and 80% levels of inhibition towards both the fungal strains, while in case of *Cuc*, 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 PWD spices used (Fig. 1).

The concentration of PWD spice sample which produced 80% level of inhibition (up to 24 days of total incubation period of 30 days) against test fungi was considered as its minimum inhibitory concentration (MIC) (Fig. 1, Table 2). It was noticed that lower concentrations of PWD spices were required to inhibit *Rs* than *Ra*. On the basis of days of inhibition produced and MIC values obtained, PWD spices can be put in the following order in terms of their decreasing antifungal activities towards *Ra* and *Rs*: *Ciz> Sya> Brn> Cuc>Als> Cul= Mep= Ocs= Trf = Zio.*

Table 2. Levels of inhibition generated by PWD spices

Spice	Fungi	Levels of Inhibiton/ Spice concentration (%, w/v)		
		40%	60%	80%
Brn	*Ra*	3.5	4.4	5.0
	Rs	2.8	3.5	4.2
Ciz	*Ra*	1.2	2.2	2.9
	Rs	0.5	0.9	1.2
Cuc	*Ra*	4.0	5.0	ND
	Rs	3.6	4.6	ND
Sya	*Ra*	1.5	2.2	2.8
	Rs	0.7	1.0	1.6

Ra: Rhizopus arrhizus, Rs: Rhizopus stolonifer, Brn: Brassica nigra, Ciz: Cinnamomum zeylanicum, Cuc: Cuminum cyminum, Sya: Syzygium aromaticum.

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Antifungal activities of EOs and WEs of spices: Results (Table 3) of impregnated paper disc method show that petri plates poured with WEs (10 µL) of tested spices did not show any growth inhibitory zones towards *Ra* and *Rs*. Same response of fungal strains was noticed towards EOs (5 µL) of *Cul, Trf* and *Zio,* and also with the control set of petri plates incorporated with DMSO (5 µL). Contrastingly, EOs of *Als, Brn, Ciz, Cuc, Mep* and *Sya* displayed distinct zones of inhibition (mm) of varying diameters towards both the fungi under observation. EO of *Brn* exhibited widest inhibitory zone measuring diameter 26.15 mm, against *Ra*, while *Sya* showed broadest zone of inhibition measuring diameter 33.20 mm towards *Rs*.

Table 3. Inhibitory activities of EOs and WEs

Spices	Zones of Inhibition (mm)					
	DMSO (5 µL)		EOs (5 µL)		WEs (10 µL)	
	Ra	*Rs*	*Ra*	*Rs*	*Ra*	*Rs*
Als	ND	ND	19.00±0.15	23.00±0.33	ND	ND
Brn	ND	ND	26.15±0.54	33.05±0.19	ND	ND
Ciz	ND	ND	17.10±0.16	21.00±0.74	ND	ND
Cuc	ND	ND	17.20±0.65	20.00±0.97	ND	ND
Cul	ND	ND	ND	ND	ND	ND
Mep	ND	ND	8.00±0.12	16.20±0.03	ND	ND
Ocs	ND	ND	ND	ND	ND	ND
Sya	ND	ND	25.00±0.36	33.20±0.28	ND	ND
Trf	ND	ND	ND	ND	ND	ND
Zio	ND	ND	ND	ND	ND	ND

Ra: Rhizopus arrhizus, Rs: Rhizopus stolonifer, ND: Not Detected, DMSO: Dimethylsulphoxide, *Als: Allium sativum, Brn: Brassica nigra, Ciz: Cinnamomum zeylanicum, Cuc: Cuminum cyminum, Cul: Curcuma longa, Mep: Mentha piperita, Ocs: Ocimum sanctum, Sya: Syzygium aromaticum, Trf: Trigonella foenum-graecum, Zio: Zingiber officinale.*

It is obvious from the diameters of growth inhibitory zones

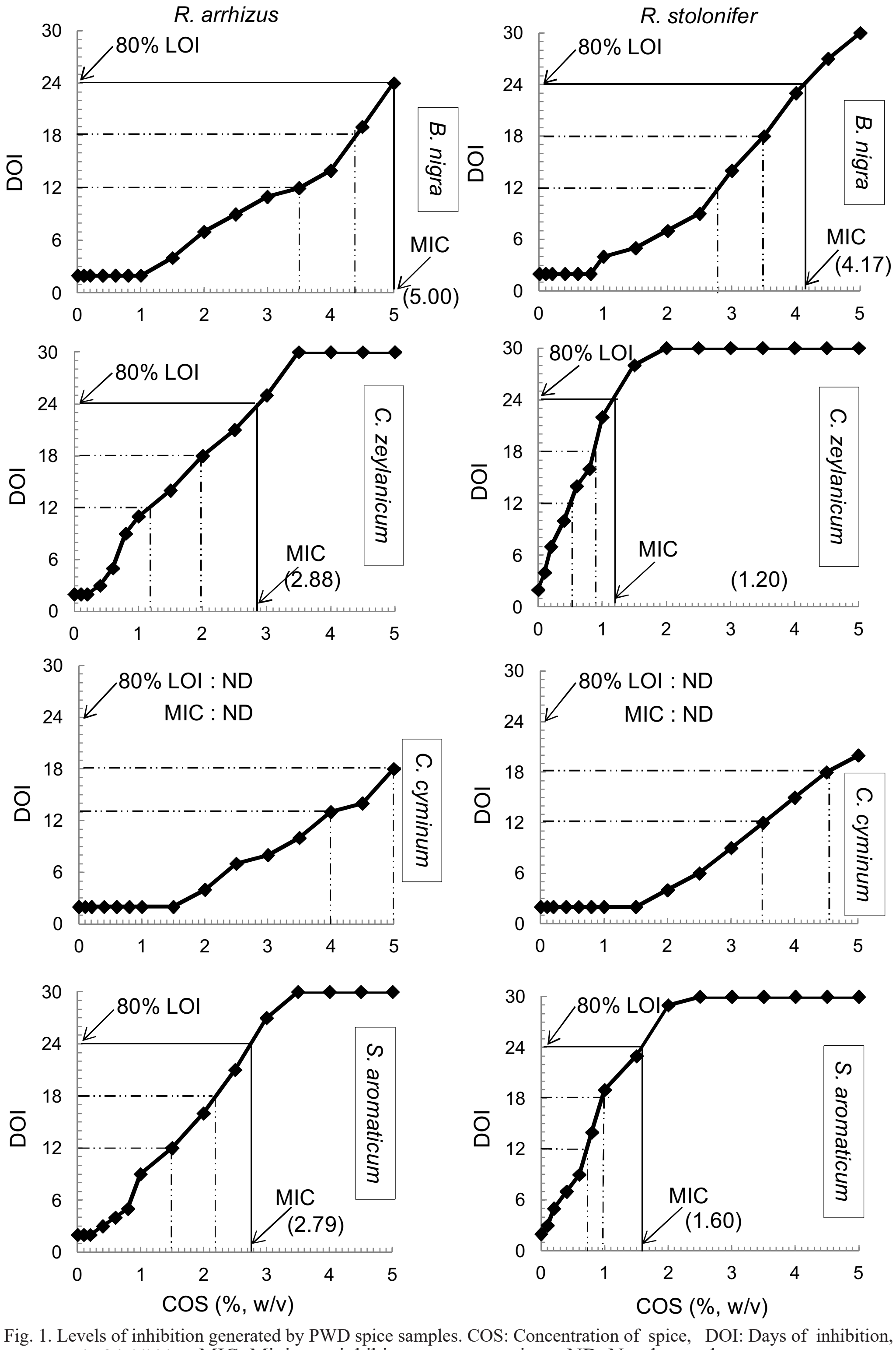

Fig. 1. Levels of inhibition generated by PWD spice samples. COS: Concentration of spice, DOI: Days of inhibition, LOI: Level of inhibition, MIC: Minimum inhibitory concentration, ND: Not detected

Table 4. Minimum inhibitory concentrations (MICs) of EOs

MICs	Spices	*Als*	*Brn*	*Ciz*	*Cuc*	*Mep*	*Sya*
DMSO	Ra	ND	ND	ND	ND	ND	ND
	Rs	ND	ND	ND	ND	ND	ND
EOs	Ra	15.62	7.81	15.62	15.62	31.25	7.81
	Rs	7.81	3.90	3.90	7.81	15.62	3.90

Ra: Rhizopus arrhizus, Rs: Rhizopus stolonifer, ND: Not Detected, DMSO: Dimethylsulphoxide, *Als: Allium sativum, Brn: Brassica nigra, Ciz: Cinnamomum zeylanicum, Cuc: Cuminum cyminum, Mep: Mentha piperita, Ocs: Ocimum sanctum, Sya: Syzygium aromaticum*

(Table 3) that *Rs* gave wider growth inhibitory zones as compared to *Ra* 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: *Ra*: *Brn> Sya> Als> Cuc> Ciz> Mep; Rs: Sya> Brn> Als> Ciz> Cuc> Mep.*

MICs of EOs (which exhibited inhibitory zones during impregnated paper disc method), were determined using broth dilution assay, and are enlisted in Table 4. Data revealed that MIC values of EOs towards *Ra* ranged from 7.81 µL/mL- 31.25 µL/mL, and towards *Rs,* MIC values varied from 3.90 µL/mL- 15.62 µL/mL. Again, it is important to point out that higher dilutions of EOs were required to inhibit the growth of *Ra* as compared to *Rs*. On the basis of MIC values obtained, effectiveness of spice EOs in descending order towards fungi can be put as: *Ra: Brn= Sya> Als= Ciz= Cuc> Mep; Rs: Brn= Ciz= Sya> Als= Cuc>Mep.*

Discussion

In present study, spice forms exhibited marked variability in inhibiting *Ra* and *Rs* in culture media. EOs of spices turned out as most potent antifungal agents, followed by PWD forms and WEs. Our findings are similar to the findings of Hetta *et al.* (2020), those elucidated that EOs and PWD forms of cinnamon, clove, and cumin, have antimicrobial activities against *Bacillus* spp., to which they further added that antimicrobial effects of EOs were more pronounced compared to the PWD forms of the same spice plant.

Among PWD spice samples tested in current trials, *Ciz* was found most potent in inhibiting fungal growth followed by *Sya, Brn, Cuc* and *Als*, whereas PWD forms of *Cul, Mep, Ocs, Trf*, and *Zio* were found altogether ineffective in arresting the growth of *Ra* and *Rs*. Our results are in agreement with some previous researches in which PWD forms of different herbs and spices, have been reported to show variable antimicrobial effects on several harmful microbes including bacteria, fungi and yeasts, like *Aspergillus* spp., *Alternaria* spp. *Bacillus subtilis, Candida albicans, Escherichia coli*, *Fusarium* spp., *Penicillium* spp., and *Rhizopus* spp. (Bhatwalkar *et al.,* 2021).

This variation in the antifungal activities shown by different spice forms in present *in vitro* study may be attributed to their volatile EOs, which in turn consist of a myriad of phytochemicals (Gutierrez-del-Rio *et al.,* 2018; Papadochristopoulos *et al.,* 2021). Therefore, in current study, more pronounced antifungal activities of PWD forms of *Brn, Ciz, Cuc,* and *Sya,* and weak antifungal effect shown by PWD *Als* during spice agar method, may be due to different structures and different modes of action of their major chemical components preferably known as bioactive components. Allicin, allyl isothiocyanate, cinnamic aldehyde, cuminic aldehyde, and eugenol are bioactive components of *Als, Brn, Ciz, Cuc,* and *Sya*, respectively (Aala *et al.,* 2014; Martinez-Pabon and Ortega-Cuadros, 2020; Patil *et al.,* 2023). Allyl isothiocyanate is an organo-sulphur compound and has been reported to show growth inhibitory effect on *C. albicans* by altering its sterol profile, blocking ergosterol biosynthesis, and by changing the expression of genes involved in the signal transduction pathway (Patil *et al.,* 2023). Allicin is also an organo-sulphur compound and was held responsible for causing disintegration and deterioration of cytoplasm parts, as well as breakdown of the cell wall, with collapse of hyphae of fungi *Trichophyton rubron* (Aala *et al.,* 2014). Cinnamic aldehyde and cuminic aldehyde are aldehydes and have shown strong inhibitory effects against *C. albicans* and *A. flavus,* respectively, by inhibiting plasma membrane ATPase and by down regulating the expression of genes involved in the synthesis of ergosterol (Xu *et al.*, 2021). Eugenol is a phenol and its inhibitory effect on *Saccharomyces cerevisiae* was noticed due to the disruption of membrane integrity, weakening of the defense system through free radical cascade-mediated lipid peroxidation and inhibition of amino acid permeases (Mak *et al.*, 2019).

Non effectiveness of PWD forms of *Cul, Mep, Ocs, Trf*, and *Zio* in present piece of work may be either due to their less amount of EOs or due to the substantial loss of volatile antifungal components during the process of drying and grinding. Other related factors responsible for variable antifungal potentials of PWD spices may include the pH, volatility, molecular weight and diffusion of antifungal components in growth medium along with type of fungal strain implicated in the study. However, exact mechanisms of action of these bioactive components towards *Ra* and *Rs* at molecular levels are not yet understood, and will remain a line of future research.

Similar reasons can be given for differential antifungal activities shown by tested EOs towards *Ra* and *Rs,* during broth dilution assay and impregnated paper disc method. Our findings were in accordance with the findings of De-Montijo-Prieto *et al.* (2021). They reported that EOs from 13 herbs, spices, fruits and vegetables exhibited variable antifungal activities towards some common food borne pathogens. Some other studies have also documented the differential antifungal activities of EOs of coriander and fennel towards fungi, namely, *A. niger, F. moniliforme, P. viridicatum, Curvularia lunata,* and *Trichoderma viride* (Liu *et al.,* 2017).

It was also noticed that *Mep* was less potent in inhibiting test fungi compared to EOs of *Als, Brn, Ciz, Cuc* and *Sya*. Least effectiveness of EO of *Mep* in present investigation could be due to its bioactive component menthol. Menthol is an alcohol and its lipophilic characteristics allow it to migrate through the extracellular medium and interact with the phospholipid membranes, causing damage to these structures and even producing permeabilization and the subsequent leakage of intracellular material, thus destabilizing the microbe (Martinez-Pabon and Ortega-Cuadros, 2020).

The inert nature of 10 WEs in current trials could be due to the insolubility of hydrophobic antimicrobial components of EOs in water. Gonelimali *et al.* (2018) also found that WEs of hibiscus and clove did not show antimicrobial activity against *C. albicans*. It was noteworthy that antifungal effect of EOs and PWD forms of spices was more pronounced against *Rs* compared to *Ra*. Though, reasons for greater susceptibility of *Rs* need further elucidation.

In conclusion, *Cul, Trf* and *Zio,* may be recognized as 'non-inhibitors'. Contrarily, *Brn, Ciz, Cuc* and *Sya,* in their two test forms, *i.e.*, EOs and PWD forms, inhibited *Ra* and *Rs* remarkably, and hence, may be considered as 'potent inhibitors' of fungal strains. Therefore, this study would help to reduce post-harvest loss of valuable horticultural crops and meet the ever increasing consumers' demand of wholesome food which is natural and free from harmful chemical additives.

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