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Evaluation of *in vitro* combinatory growth-inhibitory effects of thymol and thymoquinone against foodborne pathogens using broth volatilisation chequerboard method in vapour phase

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INTRODUCTION & AIM

Foodborne pathogens are the cause of a huge number of illnesses, hospitalizations, and deaths per year, causing loss to human health and the economy [1]. Plant-derived volatile agents offer a safe and environment-friendly alternative, with their vapours protecting food through atmospheric distribution and lowering the risk of preservative residues in food products. Combining multiple volatile antimicrobial agents can create a synergistic effect against foodborne pathogens, significantly increasing their effectiveness, while also establishing a protective antimicrobial atmosphere around the food through the release of vapours from the volatiles adsorbed onto a solid volatilisation matrix within the packaging. [2]. Therefore, there is a need to test their combinatory effects against food pathogens in the vapour phase for food preservation.

METHODOLOGY

In vitro growth-inhibitory effects of plant volatiles namely thymol and thymoquinone in combination were tested against various food pathogens such as *Bacillus cereus, Enterococcus faecalis, Escherichia coli, Listeria monocytogenes, Salmonella enterica* Typhimurium*, Shigella flexneri, Vibrio parahaemolyticus* and *Yersinia enterocolitica* using broth volatilisation chequerboard method [3]. The 96-well plates were used and various concentrations of the combinations of the compounds were prepared using automated pipetting platform Freedom EVO 100 equipped with four-channel liquid handling arm. The microbes were then inoculated on the lid and wells and incubated at 25℃ for 24h. The inhibitory effects of the vapours of the volatiles were determined by colouring using MTT assay on the lids as well as in the wells.

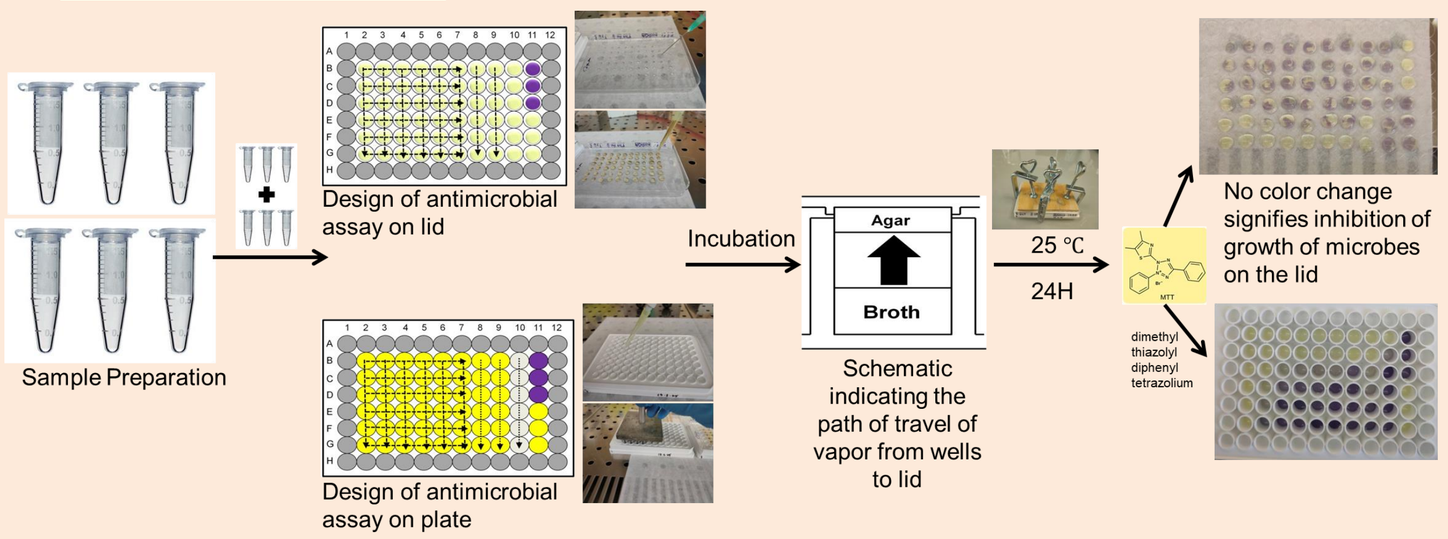

Figure 1: Pictorial representation of broth volatilisation chequerboard method.

Table 1: In vitro growth-inhibitory activity of thymol and thymoquinone against foodborne pathogens using broth volatilisation chequerboard method in vapour phase.

Microorganism	MICs alone (µg/cm³)		Thymol in combination with listed thymoquinone concentrations (µg/cm³)																					
	T	Q	+C 64		+C 32		+C 16		+C 8		+C 4		+C 2		+C 1		+C 0.5		+C 0.25		+C 0.125		+C 0.0625		+C 0.03125	
			MIC	ΣFIC	MIC	ΣFIC	MIC	ΣFIC	MIC	ΣFIC	MIC	ΣFIC	MIC	ΣFIC	MIC	ΣFIC	MIC	ΣFIC	MIC	ΣFIC	MIC	ΣFIC	MIC	ΣFIC	MIC	ΣFIC
Bacillus cereus	64	4	-	-	-	-	-	-	-	-	-	-	>2	-	>2	-	>2	-	>2	-	>2		>2	-	-	-
Enterococcus faecalis	128	8	-	-	-	-	-	-	-	-	4	0.53	4	0.28	4	0.15	4	0.09	4	0.06	4	0.04	-	-	-	-
Escherichia coli	64	32	-	-	-	-	>16	-	>16	-	>16	-	>16	-	>16	-	>16	-	-	-	-	-	-	-	-	-
***Escherichia coli* O157:H7**	32	2	-	-	-	-	-	-	-	-	-	-	-	-	1	0.52	1	0.27	1	0.15	1	0.08	1	0.05	1	0.04
Listeria monocytogenes	64	2	-	-	-	-	-	-	-	-	-	-	-	-	1	0.51	1	0.26	1	0.14	1	0.07	1	0.04	1	0.03
***Salmonella enterica* Typhimurium**	128	128	>64		>64	-	>64	-	>64	-	>64	-	>64	-	-	-	-	-	-	-	-	-	-	-	-	-
Shigella flexneri	128	128	64	1	64	0.75	64	0.62	64	0.56	64	0.53	64	0.51	-	-	-	-	-	-	-	-	-	-	-	-
Vibrio parahaemolyticus	128	128	64	1	64	0.75	64	0.62	64	0.56	64	0.53	64	0.51	-	-	-	-	-	-	-	-	-	-	-	-
Yersinia enterocolitica	128	16	-	-	-	-	-	-	>8	-	>8	-	8	0.12	8	0.08	8	0.06	8	0.05	-	-	-	-	-	-

T: Thymol; MIC: minimum inhibitory concentration – the values are expressed as an average from three independent experiments, each performed in triplicate (rounded to integers); Q: Thymoquinone; -: Not Tested; ΣFIC: sum of fractional inhibitory concentrations; The combinatory effect is evaluated as follows: synergy ΣFIC ≤0.5; additive ΣFIC > 0.5 and ≤ 1 (rounded to 2 decimal places)

RESULTS & DISCUSSION

The results obtained in this study showed ΣFIC values in the range of 0.031-0.281 in vapour phase indicating synergistic interactions of the compound for *E. coli*, *E. faecalis*, and *L. monocytogenes*. They also exhibited several additive effects with ΣFIC values of 0.515-1 in vapour phase for *S. e. Typhimurium*, *S. flexneri*, and *V. parahaemolyticus*. While *B. cereus* and *Y. enterocolitica* were less susceptible to the combination of thymol and thymoquinone and showed ΣFIC values >0.052 in vapour phase.

CONCLUSION

These results indicated greater effects of lower concentration of the compounds in inhibiting the growth of pathogens when used in combination, unlike lesser effects when used alone. Therefore, the combination of thymol and thymoquinone shows promise as antibacterials for their applications as food preservatives against food pathogens, producing a greater effect. However, further research on their safety, organoleptic properties and efficacy in food models is very much needed before their incorporation into practical application.

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