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Silver nanoparticles-essential oils combined treatments to enhance the antibacterial and antifungal properties against foodborne pathogens and spoilage microorganisms

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ABSTRACT

Plant-derived essential oils (EOs) and commercial silver nanoparticles (AgNPs) were tested to evaluate their antibacterial and antifungal efficiency against two pathogenic bacteria (*Escherichia coli* O157:H7 and *Salmonella* Typhimurium) and three spoilage fungi (*Aspergillus niger*, *Penicillium chrysogenum*, and *Mucor circinelloides*). A broth microdilution assay was used to determine the minimal inhibitory concentration (MIC) of EOs and AgNPs. In the MIC assay, the cinnamon EO, Mediterranean formulation, citrus EO and spherical-shaped silver nanoparticles (AgNPs) (AGC 1, AGC 0.5, AGPP and AGPPH) showed moderate to high antibacterial and antifungal properties, with MIC ranging from 7.8 to 62.5 ppm for AgNPs and 312.5–1250 ppm for EOs against the tested bacteria and fungi. The possible interaction between the EOs and the AgNPs was determined using a checkerboard method by evaluating fractional inhibitory concentration (FIC) values. The combination of two or more EOs and AgNPs (Active combination 1: AGPPH+cinnamon EO, Active combination 2: AGC 0.5+Mediterranean formulation+citrus EO, Active combination 3: AGPP+cinnamon EO+Asian formulation+lavang EO) showed synergistic effects (FIC <1.0) against all tested bacteria and fungi. A modified Gompertz model was used to evaluate growth parameters including maximum colony diameter (A), maximum growth rate (V_m), and lag phase (λ), under the three active combinations suggested by the checkerboard method using a vapor assay. The three active combinations 1, 2 and 3 reduced the growth rate and maximum colony diameter of *E. coli*, *S.* Typhimurium, *A. niger*, *P. chrysogenum*, and *M. circinelloides*, and extended their lag phase from 1 to 5 days. In *in situ* tests with inoculated rice, the three active combinations showed a significant reduction of all tested bacteria and fungi at 27 °C for 28 days.

1. Introduction

Rice (*Oryza sativa* L.) is an important cereal crop with a worldwide annual production of over 600 million tons annually [1]. Stored rice is prone to deterioration under storage conditions (temperature, relative humidity, moisture contents) that promote bacterial and fungal growth [1]. The most common spoilage fungi are *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., and these fungi may produce mycotoxins which are highly toxic to human and animals. Spoilage fungi can also cause grain discoloration, chemical and nutritional changes, and reduced germination [2,3]. Rice is susceptible to contamination by bacteria such as *Escherichia coli*, *Salmonella* Typhimurium, and *Listeria monocytogenes* as well [4].

Natural food extracts such as plant essential oils (EOs) are a safe alternative to the use of synthetic chemical food preservatives. Plant EOs are widely known to have antibacterial, antifungal, antiviral, insecticidal, and antioxidant properties [3,5–8]. EOs are secondary metabolites of plants with a complex mixture of volatile active compounds especially monoterpenes and sesquiterpenes, and their oxygenated derivatives (alcohols, aldehydes, esters, ethers, ketones, phenols, and oxides) [6,8,

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Table 1
Name and compositions/chemical components of the essential oils (EOs).

Name of antibacterials/ antifungals	Compositions/chemical components
Mediterranean formulation	origanum oil, black pepper oil, capsicum oleoresin (OR), garlic oil, pimento berry oil, lemongrass oil, citral oil.
Asian formulation	lemongrass, citral oil, pimento berry oil, ginger oleoresin, Indian celery seed oil, black pepper oleoresin, cumin oil, nutmeg oil, coriander seed oil, caraway oil, capsicum oleoresin, garlic oil.
Cinnamon EO	cinnamaldehyde, cinnamyle acetate, β-carryophyllene, -cymene.
Citrus EO	Sweet orange (limonene, myrcene).
Lavang EO	beta-caryophyllene, alpha-humulene, eugenol, eugenyle acetate.

9]. More than 300 EOs have found application in the food, pharmaceutical, sanitary, or cosmetic industries and they are generally recognized as safe (GRAS) by the US Food and Drug Administration (FDA) and the European Commission. However, perceivable taste and aroma changes caused by EOs at high concentrations may limit their application in many food products. The combination of two or more antimicrobial/antifungal EO agents may provide synergistic and increased activity at low concentrations to avoid this limitation [8,10–12].

Silver nanoparticles (AgNPs) are increasingly used in the medical, food, healthcare, and industrial fields due to their unique physical and chemical properties, including their well-known antimicrobial activity at low concentrations [13,14]. The microbicidal effect of AgNPs has three possible modes of action: (1) small size AgNPs can bind to the cell membrane surface and disrupt its functions, permeability, and respiration, (2) AgNPs as a weak acid (Lewis acid) can interact with compounds containing sulfur and phosphorus (DNA, proteins) after penetration into microbial cells, and (3) The release of Ag^{+} in the presence of oxygen can interacti with negatively charged cell membranes which enhances other microbicidal effects [15–18].

In the present study, the antibacterial and antifungal activity of five EOs and four spherical silver nanoparticle types (AGC 1, AGC 0.5, AGPP and AGPPH) were evaluated against pathogenic bacteria and spoilage fungi by determining (i) the minimal inhibitory concentration (MIC), (ii) possible synergistic antibacterial and antifungal interaction among EOs and AgNPs, (iii) the growth kinetics of bacteria and fungi in the presence of selected EO/AgNP combinations, and (iv) the *in situ* antibacterial and antifungal effectiveness of select EO/AgNP combinations in rice during storage.

2. Materials and methods

2.1. Materials

The essential oils (EOs) of Asian formulation and Mediterranean formulation were purchased from BSA (Montreal, Quebec, Canada), and cinnamon EO, citrus EO and lavang EO were obtained from Zayat Aroma (Bromont, QC, Canada). The Mediterranean formulation composed of seven oils (origanum oil: black pepper oil: capsicum oleoresin (OR): garlic oil: pimento berry oil: lemongrass oil: citral oil (5.85: 0.25: 0.25: 0.25: 1.4: 1.15: 0.85)) and Asian formulation composed of twelve oils (lemongrass: citral oil: pimento berry oil: ginger oleoresin: Indian celery seed oil: black pepper oleoresin: cumin oil: nutmeg oil: coriander seed oil: caraway oil: capsicum oleoresin: garlic oil (1.78: 1.2: 0.57: 0.65: 0.47: 0.47: 0.22: 1.94: 0.8: 0.29: 0.6: 1)). The compositions/chemical components of EOs were mentioned in Table 1. Four different types of commercial silver nanoparticle (AgNPs) containing formulations namely as AGC 1, AGC 0.5, AGPP, and AGPPH were provided by NanoBrand (Bernard-Belleau, Laval, Quebec, Canada). Tween 80 (emulsifier), NaCl (for saline water) and glycerol were purchased from Sigma-Aldrich Ltd. (St. Louis, Missouri, United States), the stabilizer polyvinylpyrrolidone (PVP) (average molecular weight 40,000) and chitosan (8% deacetylated Chitosan) were purchased from Alfa Aesar (Ward Hill, Massachusetts, United States), and polyethylene glycol (average molecular weight 600) was bought from Acros Organics (Fair Lawn, New Jersey, United States). The potato dextrose broth (PDB) and tryptic soy broth (TSB) was purchased from Alpha Biosciences Inc. (Baltimore, MD, USA) and BD, Franklin Lakes, NJ, USA), respectively.

2.2. Preparation of antimicrobial and antifungal compounds

The plant-derived essential oils (EOs) Mediterranean formulation, citrus, cinnamon, lavang, and Asian formulation were tested as natural antibacterial and antifungal agents. The oil-in-water (O/W) emulsions were prepared using 2% (v/v) EO, 1% (w/v) Tween 80, and 97% distilled water (w/w), and were homogenized for 1 min at 15,000 rpm using Ultra Turrax (TP18/1059 homogenizer) before use. All the samples containing silver nanoparticles (AGC 1, AGC 0.5, AGPP, and AGPPH) had a silver concentration of 1000 ppm. AGPP and AGPPH samples were contained silver nanoparticles stabilized by polyvinylpyrrolidone and polyethylene glycol with different pH (AGPP: pH = 3; AGPPH: pH = 6). The nanoparticles containing formulations AGC 0.5 and AGC 1 were dispersed in 0.5% and 1% of chitosan, respectively.

2.3. Preparation of bacteria/fungi cultures and assay media

The bacteria (*S.* Typhimurium SL 1344 and *E. coli* O157:H7 NT 1931) and fungi (*A. niger* ATCC 1015, *P. chrysogenum* ATCC 10106, and *M. circinelloides* ATCC 56649) were collected from the American Type Culture Collection (ATCC), except, *E. coli* O157:H7. *E. coli* O157:H7 was collected from United States Department of Agriculture (USDA), Albany, CA, United States. All bacterial and fungal strains were stored at −80 °C in 10% (v/v) glycerol on TSB and in PDB, respectively [19,20]. Before each experiment, the stock cultures were propagated through two consecutive growth cycles in TSB at 37 °C for 24 h (bacteria) or in PDB at 28 °C for 48 h (fungi) [19,20,45]. The bacterial cultures were recovered by centrifugation and washed with 0.85% (w/v) saline water to obtain the desired pathogen concentrations for inoculation. However, the fungal cultures were pre-cultured in sterile PDA media for 2–4 days at 28 °C and the spores were collected from the culture media using sterile saline water and filtered. Final bacterial and fungal spore culture concentrations were adjusted approximately 1×10^5 CFU/mL or 1×10^5 spores/mL respectively, for all *in vitro* and *in situ* experiments [19,20,45].

2.4. Minimal inhibitory concentration (MIC)

A modified broth microdilution method was used to determine the minimum inhibitory concentration (MIC) of the AgNPs and EOs as described by Turgis et al. [8]. The AgNPs and EOs were classified into three distinct groups based on MIC values, and the groups were i) highly effective (<625 ppm) ii) moderately effective (625–1250 ppm), and iii) less effective (>1250 ppm). A 100 μL aliquot of a 2-fold serial dilution (from 0.48 to 500 ppm) of AgNPs and from 156 to 10,000 ppm for EO suspensions were prepared and deposited in each well of a 96-well microplate (Sarstedt, St-Leonard, QC, Canada) using TSB for bacteria or PDB for fungi. Each well was then inoculated with 100 μL of a pathogen at a concentration of 10^5 CFU/mL (bacteria) or 10^5 spores/mL (fungi) and incubated for 24 h at 37 °C or 48 h at 28 °C. In the 96-well plate, one well served as a positive control containing the pathogen and TSB/PDB, and a negative control contained no pathogen. Microbial growth was evaluated using an Ultra Microplate Reader (Biotek Instruments, Winooski, VT, USA) by measuring the optical density (OD) at 595 nm. The MIC was defined as the lowest concentration of the AgNPs or EOs suspension that completely inhibited the bacterial and fungal growth.

2.5. Synergistic interactions of essential oils and silver nanoparticles

Interactions of EOs and AgNPs were evaluated using a checkerboard microdilution test. The checkerboard tests were performed using 96-well microplates to measure the fractional inhibitory concentration (FIC) index of EOs and AgNPs against each bacterium and fungus [8,12,20,21]. For selected double combinations, the checkerboard test was used against all tested bacteria and fungi with two-fold dilutions of twenty treatments, including AGC 0.5+Mediterranean formulation, AGC 0.5+cinnamon EO, AGC 0.5+Asian formulation, AGC 0.5+citrus EO, AGC 0.5+lavang EO, AGPP+cinnamon EO, AGPP+Asian formulation, AGPP+lavang EO, AGPP+citrus EO, AGPP+Mediterranean formulation, AGPPH+Mediterranean formulation, AGPPH+cinnamon EO, AGPPH+Asian formulation, AGPPH+citrus EO, AGPPH+lavang EO, AGC 1+Mediterranean formulation, AGC 1+cinnamon EO, AGC 1+Asian formulation, AGC 1+citrus EO, AGC1+lavang EO. A volume of 100 μL of bacterial/fungal suspension (containing 10^5 CFU/mL) was added to the each well of the microplate. The microplates were then incubated in a shaking incubator at 37 °C (bacteria)/and 28 °C (fungi) for 24 (bacteria) or 48 (fungi) hours, respectively. The corresponding readings were taken with a microplate reader (BioTek, ELx800™) at 595 nm.

The dual combinations of EOs and AgNPs which showed synergy were combined with a third component for assessing 3-way synergistic interactions using a three-dimensional checkerboard method [22]. For the three-dimensional checkerboard method, nine combinations including (AGC 0.5+Mediterranean formulation) and citrus EO, (AGC 0.5+cinnamon EO) and Asian formulation, (AGPP+cinnamon EO) and lavang EO, (AGPP+cinnamon EO) and Asian formulation, (AGPP+Asian formulation) and lavang EO, (AGC 1+cinnamon EO) and Asian formulation, (AGC 1+cinnamon EO) and citrus EO, (AGPP+cinnamon EO+Asian formulation) and lavang EO were selected for evaluation. The well of the microplate containing the nutrient medium (TSB or PDB) with bacterial or fungal inoculum served as a positive control and the well without inocula (containing active EO and Ag components only) served as a negative control. All assays were performed in triplicate. The FIC values for 2 (Eq. (1)), 3 or more (Eq. (2)) EOs, and AgNPs were calculated using Eq. (1).

$$FIC = FIC1 + FIC2 \quad \text{Eq. (1)}$$

Where, FIC1 = (MIC1 combined/MIC1 alone) and FIC2 = (MIC2 combined/MIC2 alone)

$$FIC = FIC\ (1+2) + FIC3 \quad \text{Eq. (2)}$$

Where, FIC (1 + 2) = (MIC1+2 combined/MIC1+2 alone) and FIC3 = (MIC3 combined/MIC3 alone).

A FIC <1.0 was interpreted as a synergistic effect, FIC = 1 represented as additive effect, FIC >1 represented an antagonistic effect.

The three active combinations of EOs and AgNPs such as AGPPH + cinnamon EO, AGC 0.5+Mediterranean formulation + citrus EO, and AGPP + cinnamon EO + Asian formulation + lavang EO named active combination 1, 2 and 3, respectively, were selected for further tests because of their synergistic effect against all tested pathogens.

2.6. Vapor contact assays

An inverted lid technique was used to test the efficacy of active combinations in a volatile state for food packaging applications following methods in Refs. [20,23]. Briefly, a 10 μL aliquot bacterial or fungal suspension (1×10^5 CFU/mL or 1×10^5 spores/mL) was placed in the center of the TSA plate (Trypto Soy Agar) for bacteria and PDA plate (Potato Dextrose Agar) for fungi and were dried in a laminar flow hood under aseptic conditions at room temperature for 30 min. Sterile filter paper (10 mm diameter) was placed at the center of the upper lid of the plate. A quantity of 10 μL of each active combination was added at the center of individual paper filters. A growth control was prepared in parallel to ensure that viable microorganisms were present. The Petri dishes were incubated at 37 °C for bacteria and 27 °C for fungi for 8 days. Every test was performed in triplicate. Bacterial and fungal growth modeling was fitted using a modified Gompertz model as reported by Char et al. [24].

2.7. Bacterial and fungal growth model and statistical analysis

The growth model and parameters for each bacteria and fungi under the vapor treatment of active combinations on pure nutrient media (TSA or PDA) and compared with control plate (without active combinations) and fitted using the modified Gompertz equation [23,24]:

$$ln\frac{Dt}{D0} = A.exp\left\{-exp\left[\frac{Vm.e}{A}\right](\lambda - t) + 1\right]\right\} \quad \text{Eq. 3}$$

Where D_t is the average colony diameter (cm) at time t (day); D_0 is the average colony diameter (cm) at the initial time (day 0); A stands for the maximum growth achieved during the stationary phase; Vm is the maximum specific growth rate (1/day); λ is the lag phase (day).

The one-way analysis of variance (ANOVA) and Duncan test at α = 0.05 was performed for statistical analysis using SPSS software (IBM Corporation, Somers, NY, USA). Three replicates were performed for each treatment and the differences between mean values at $P \leq 0.05$ were considered significant.

2.8. In situ antibacterial and antifungal efficiency of active combinations in rice

An *in situ* test was performed in packaged rice to evaluate the antibacterial and antifungal properties against the pathogenic bacteria (*E. coli* O157:H7, *S.* Typhimurium) and fungi (*A. niger*, *P. chrysogenum*, and *M. circinelloides*) according to Hossain et al. [3]. A volume of 200 μL of bacteria or fungi (1×10^5 CFU/mL for bacteria or 1×10^5 spores/mL for fungi) was inoculated in 50 g of sterile rice (Super quality basmati rice, Pitfield Ville St, Laurent, Quebec, Canada). A sterile sponge cube (5 × 5 × 5 cm) containing a volume of 50 μL of the active combination was placed inside a sterile plastic cup. A muslin screen was used to cover the cup to prevent contact between rice grains and the active combinations and placed them inside the rice. The samples were containing rice and inocula denoted as control groups (without active combinations). The samples were incubated for 28 days at 37 °C and 27 °C for bacteria and fungi, respectively, and the microbiological analyses were performed at 1, 7, 14, 21, and 28 days of storage.

2.9. Microbiological analysis

The microbiological analyses of stored rice were carried out using a standard method International Commission of Microbiological Specification on Foods (ICMSF) [25]. A volume of 20 mL of sterile peptone water (0.1%, w/v) was added in 10 g of rice and homogenized for 60 s at 260 rpm by a Lab-blender 400 stomacher (Laboratory Equipment, London, UK). A serial dilution (from 10^{-1} to 10^{-6}) of the homogenized sample was prepared and a 0.1 mL of diluted sample was inoculated onto TSA media for bacteria and PDA media fungi. Then the plates were incubated at 37 °C and 27 °C for 24 h (bacteria) and 48 h (fungi), and the microbial colonies were counted followed by Begum et al. [4]. Each experiment was performed in triplicate.

3. Results and discussion

3.1. Minimal inhibitory concentration (MIC)

The antibacterial and antifungal effects of EOs and AgNPs in terms of MICs against pathogenic bacteria (*E. coli* O157:H7 and *S.* Typhimurium)

Table 2
Minimal inhibitory concentration (MIC, ppm) of AgNPs and EOs against *E. coli* O157:H7, *S.* Typhimurium, *A. niger*, *P. chrysogenum*, *M. circinelloides*.

Antibacterials/Antifungals	Minimal Inhibitory Concentration (ppm)				
	E. coli O157:H7	*S.* Typhimurium	*A. niger*	*P. chrysogenum*	*M. circinelloides*
AGC 1	7.8	7.8	15.6	31.2	31.2
AGC 0.5	7.8	7.8	7.8	62.5	31.2
AGPP	15.6	15.6	7.8	7.8	15.6
AGPPH	7.8	7.8	7.8	62.5	7.8
Cinnamon EO	1250	1250	625	312.5	1250
Asian formulation	5000	5000	1250	612	5000
Mediterranean formulation	1250	1250	1250	1250	1250
Citrus EO	1250	1250	625	625	625
Lavang EO	5000	5000	2500	2500	1250

and spoilage fungi (*A. niger*, *P. chrysogenum*, and *M. circinelloides*) are presented in Table 2. Results showed that among all tested AgNPs (AGC 1, AGC 0.5, AGPP, and AGPPH) have significant antibacterial activity against all tested pathogenic bacteria (*E. coli* O157:H7 and *S.* Typhimurium) with MIC values ranging from 7.8 to 62.5 ppm. The AGC 1, AGC 0.5, and AGPPH formulations showed the highest antibacterial activity against *E. coli* O157:H7 and *S.* Typhimurium, with MIC values of 7.8 ppm. The AGPP formulation showed a significant inhibitory effect against *E. coli* O157:H7 and *S.* Typhimurium, with an MIC of 15.6 ppm. All tested AgNPs showed strong antifungal activity against *A. niger*, *P. chrysogenum*, and *M. circinelloides*. AGPP showed the highest antifungal activity against *A. niger* and *P. chrysogenum*, with MIC values of 7.8 ppm. AGPPH exhibited the highest antifungal activity against *A. niger* and *M. circinelloides*, with MIC values of 7.8 ppm. AGC 0.5 was most effective against *A. niger* (MIC of 7.8 ppm).

The cinnamon EO, Mediterranean formulation, and citrus EO showed moderate antibacterial activity (MIC of 1250 ppm) against *E. coli* O157:H7 and *S*. Typhimurium. Similarly, Mith et al. [26] also found cinnamon EO, oregano EO, clove, and lemongrass EOs had antibacterial activity against pathogenic bacteria *E. coli* O157:H7, *S.* Typhimurium and *Listeria monocytogenes*. Cinnamon EO showed highly effective antifungal activity whose MIC value was 312.5 ppm against *P. chrysogenum*, while cinnamon EO showed moderate antifungal activities against *A. niger* and *M. circinelloides*. The citrus EO showed moderately effective antifungal activity (MIC of 625 ppm) against all tested fungi.

Silver nanoparticles (AgNPs) have strong antibacterial and antifungal properties and they are widely used in the food industry as an antimicrobial agent within FDA recommended limits [27–29]. AgNPs have higher bactericidal efficacy toward Gram-negative bacteria due to their thinner cell wall, while EOs are more effective against Gram-positive bacteria. AgNPs can create pits on the cell surface of microorganisms which can lead to cell damage; they can also inhibit the production of microbial proteins and enzymes by disrupting the ribosomal activities of the bacterial cell. Moreover, AgNPs are also commonly used as antifungal agents to treat resistant fungi [27,28]. Generally, the bioactivities (e.g., cellular uptake, cellular activation intercellular distribution) of the nanoparticles depend on their size, shape, surface charge, functionalization, and core structure. In the current study, we worked with spherical and small-sized AgNPs (3–45 nm) which release Ag^+ faster, and thus leading higher bactericidal and antifungal effects due to higher concentrations of silver ions [30,31]. Martinez-Castanon and co-authors found spherical shaped AgNPs that were 7, 29 and 89 nm in diameter all exhibited strong antibacterial activity against *E. coli* [32]. Helmlinger et al. [30] demonstrated the role of AgNPs shape on antibacterial activities against *Staphylococcus aureus.* They found nanoplatelets (20–60 nm) exhibit the highest toxicity, followed by nanospheres (diameter 40–70 nm and 120–180 nm), and finally nanocubes (140–180 nm), and attributed this pattern to the effects of surface area and dissolution rate of particles, i.e., spherical and nanoplatelet shaped AgNPs have the highest specific area and formed more Ag^+ ions [17,30].

Table 3
Fractional inhibitory concentration (FIC) indices of Ag-NPs in combination with the selected EOs.

Combination of EOs/AgNPs	*E. coli* O157:H7		*S.* Typhimurium		*A. niger*		*P. chrysogenum*		*M. circinelloides*	
	FIC	Act[1]	FIC	Act[1]	FIC	Act[1]	FIC	Act[1]	FIC	Act[1]
AGC 0.5+Mediterranean formulation	1.24	AG	0.74	S	0.49	S	0.74	S	0.74	S
AGC 0.5+cinnamon EO	0.53	S	0.49	S	0.56	S	0.99	S	0.62	S
AGC 0.5+Asian formulation	0.56	S	1.49	AG	0.75	S	1.24	AG	0.49	S
AGC 0.5+citrus EO	1.5	AG	0.99	S	0.53	S	0.49	S	0.74	S
AGC 0.5+lavang EO	1.25	AG	0.49	S	0.62	S	1.12	AG	0.99	S
AGPP+cinnamon EO	0.75	S	0.37	S	0.31	S	1.00	AD	0.62	S
AGPP+Asian formulation	0.28	S	0.31	S	0.62	S	0.55	S	0.49	S
AGPP+lavang EO	1.00	AD	0.49	S	0.75	S	0.75	S	0.5	S
AGPP+citrus EO	4.12	AG	0.75	S	2.25	AG	0.50	S	0.37	S
AGPP+Mediterranean formulation	0.5	S	1.00	AD	1.12	AG	0.62	S	0.37	S
AGPPH+Mediterranean formulation	1.12	AG	0.74	S	1.00	AD	1.12	AG	1.00	AD
AGPPH+cinnamon EO	0.49	S	0.37	S	0.37	S	0.75	S	0.62	S
AGPPH+Asian formulation	0.62	S	0.56	S	1.12	AG	1.00	AD	2.24	AG
AGPPH+citrus EO	0.62	S	0.49	S	1.25	AG	0.5	S	1.00	AD
AGPPH+lavang EO	2.25	AG	0.49	S	0.50	S	0.74	S	0.75	S
AGC 1+Mediterranean formulation	1.00	AD	0.74	S	1.00	AD	2.06	AG	1.00	AD
AGC 1+cinnamon EO	0.53	S	0.74	S	0.56	S	0.74	S	1.00	AD
AGC 1+Asian formulation	0.37	S	0.49	S	1.00	AD	0.56	S	2.24	AG
AGC 1+citrus EO	0.74	S	0.99	S	0.31	S	0.99	S	0.25	S
AGC 1+lavang EO	2.24	AG	0.49	S	0.74	S	0.74	S	0.37	S

Act[1]: activity; FIC <1.0: synergic effect (S); FIC = 1.0: additive effect (AD); FIC >1.0: antagonistic effect (AG).
$^{FIC=FIC\ Ag-NPs}{}_{+}{}^{FIC}$ EOs; $^{FIC\ Ag-NPs=MIC\ Ag-NPs}$ combined to $EO^{/MIC}$ Ag-NPs alone; ^{FIC} EOs$=^{MIC}$ EO combined to Ag-$NPs^{/MIC}$ EOs alone; FIC <1.0: synergic effect (S); FIC = 1.0: additive effect (AD); FIC >1.0: antagonistic effect (AG).

Table 4
Fractional inhibitory concentration (FIC) indices of three or more combinations of EO and AgNPs.

Combination of EOs/AgNPs	*E. coli* O157: H7		*S.* Typhimurium		*A. niger*		*P. chrysogenum*		*M. circinelloides*	
	FIC	Act[1]	FIC	Act[1]	FIC	Act[1]	FIC	Act[1]	FIC	Act[1]
(AGC 0.5+Mediterranean formulation)/citrus EO	0.24	S	0.53	S	0.34	S	0.71	S	0.32	S
(AGC 0.5+cinnamon EO)/Asian formulation	2.50	AG	1.12	AG	1.56	AG	0.58	S	0.99	S
(AGPP+cinnamon EO)/lavang EO	0.37	S	0.53	S	0.76	S	0.34	S	0.71	S
(AGPP+cinnamon EO)/Asian formulation	0.62	S	0.58	S	0.32	S	0.74	S	0.34	S
(AGPP+Asian formulation)/lavang EO	2.66	AG	5.01	AG	1.0	AD	1.21	AG	2.44	AG
(AGC 1+cinnamon EO)/Asian formulation	0.58	S	0.99	S	1.23	AG	2.01	AG	1.0	AD
(AGC 1+cinnamon EO)/citrus EO	0.99	S	0.43	S	1.72	AG	2.56	AG	2.38	AG
(AGPP+cinnamon EO+Asian formulation)/lavang EO	0.74	S	0.99	S	0.99	S	0.82	S	0.51	S

Act[1]: activity; FIC <1.0: synergic effect (S); FIC = 1.0: additive effect (AD); FIC >1.0: antagonistic effect (AG).

The bioactivity of EOs mainly depends on the mixture of chemical components, their functional groups, and concentrations [5,14]. The cinnamon EO, Mediterranean formulation, citrus EO, lavang EO, and Asian formulation contain cinnamaldehyde (63%), carvacrol (46%) and thymol (14%), limonene (95%), eugenol (>85%) and geraniol (45%) and neral (32%), respectively [26,33,34]. High antifungal activity of oregano EO against *A. niger* and *P. chrysogenum* (MIC of 62.5 ppm) supports the findings of Hossain et al. [20]. The phenolic compounds carvacrol and thymol (60–74%) found in oregano EO are mainly responsible for its bioactivity [12,35,36]. EOs targets the fungal cell membranes, thus increasing cell permeability which leads to loss of cellular contents. Moreover, EOs disrupt the mitochondrion of fungi causing energy depletion, inhibiting respiration, and disrupting aflatoxin biosynthesis by inhibiting the synthesis of DNA and transcription genes [6,37,38].

3.2. Synergistic interactions of essential oils and silver nanoparticles

The antibacterial and antifungal effects of combined AgNPs and EOs by the checkerboard method against two foodborne bacteria (*E. coli* O157:H7, *S.* Typhimurium) and three spoilage fungi (*A. niger*, *P. chrysogenum*, and *M. circinelloides*) are presented in Tables 3 and 4. The selection of EOs and AgNPs was based on the high efficiency (low MIC) values. The silver nanoparticles AGC 0.5, AGPP, AGC 1 and AGPPH, and EOs of Mediterranean formulation, cinnamon EO, Asian formulation, lavang EO, and citrus EO was selected for the checkerboard tests against *E. coli* O157:H7, *S.* Typhimurium, *A. niger*, *P. chrysogenum*, and *M. circinelloides*. The dual combination between AGC 0.5+cinnamon EO, AGPP+Asian formulation EO, AGPPH+cinnamon EO, AGC 1+citrus EO, AGPP+cinnamon EO, AGPPH+Asian formulation, AGPPH+citrus EO, AGC1+cinnamon EO, and AGC 1+Asian formulation exhibited the highest synergistic activity against *E. coli* O157:H7 and *S.* Typhimurium (FIC <1). The mixture of AGC 0.5+Asian formulation as well as AGPPH+Mediterranean formulation showed synergistic activity against *E. coli* O157:H7 and *S.* Typhimurium (FIC <1), respectively. The combination of AGC 0.5+Mediterranean formulation, AGC 0.5+lavang EO, AGPP+lavang EO, AGPP+citrus EO, AGPPH+lavang EO, AGC 1+Mediterranean formulation, and AGC 1+lavang EO showed synergistic activity against *S.* Typhimurium (FIC <1) only.

The mixture of AGC 0.5+Mediterranean formulation, AGC 0.5+cinnamon EO, AGC 0.5+citrus EO, AGPP+Asian formulation, AGPP+lavang EO, AGPPH+cinnamon EO, AGPPH+lavang EO, AGC 1+citrus EO, AGC 1+lavang EO showed synergy against all tested fungal species such as *A. niger*, *P. chrysogenum* and *M. circinelloides* having FIC values below 1.0. The mixtures of AGPP+Mediterranean formulation and AGPP+citrus EO showed synergy against both *P. chrysogenum* and *M. circinelloides* (FIC <1.0), however, antagonistic activity against *A. niger* (FIC >1.0). The mixtures of AGC 0.5+Asian formulation, AGC 0.5+lavang EO, AGPP+cinnamon EO showed synergistic activity against both *A. niger* and *M. circinelloides*. The combination of AGC 1+cinnamon EO showed a synergistic effect against *A. niger* only. The AGPPH with citrus EO, AGC 1 with cinnamon EO, AGC 1 with Asian formulation exerted synergy only against *P. chrysogenum* (Table 3).

Combining antimicrobial and antifungal agents showing synergistic effects and thereby reducing the required concentration of active compounds may have several advantages including slower development of resistance and minimizing any undesirable organoleptic effects in foods. Oregano EO (*Origanum vulgare*) combined with AgNPs showed synergistic activity against resistant *E. coli* with bactericidal effects [39]. The interactions between two or more active compounds can be influenced by the type of the antibacterial/antifungal component, their concentrations, the microbial strain, and their size and shape [40]. In some cases, the plant extract may aggregate AgNPs in the mixture which may change the size and shape of the nanoparticles and could reduce the antibacterial and antifungal effects [41].

The results of antibacterial and antifungal effects of triple combinations of EO-AgNP in combination with other EOs were evaluated by the checkerboard method and the results are presented in Table 4. The triple combinations of (AGC 0.5+Mediterranean formulation) with citrus EO, (AGPP+cinnamon EO) with lavang EO, (AGPP+cinnamon EO) with Asian formulation, (AGC 1+cinnamon EO) with Asian formulation, (AGC 1+cinnamon EO) with citrus EO, and (AGPP+cinnamon EO+Asian formulation) with lavang EO showed synergistic effects against both *E. coli* O157: H7 and *S.* Typhimurium (FIC <1.0) (Table 4).

For fungi, the triple combinations of (AGC 0.5+Mediterranean formulation) with citrus EO, (AGPP+cinnamon EO) with lavang EO, (AGPP+cinnamon EO) with Asian formulation, and (AGPP+cinnamon EO+Asian formulation) with lavang EO were effective against *A. niger*, *P. chrysogenum*, and *M. circinelloides*. The triple combination of (AGC 0.5+cinnamon EO) with Asian formulation showed synergistic antifungal activity against *P. chrysogenum* and *M. circinelloides*, while an antagonistic activity was observed for (AGC 0.5+cinnamon EO) with Asian formulation against only *A. niger* (FIC >1.0) (Table 4).

The current study showed that AgNPs (AGPPH) with cinnamon EO have synergistic activities against pathogenic bacteria (*E. coli* O157:H7 and *S.* Typhimurium) and fungi (*A. niger*, *P. chrysogenum*, and *M. circinelloides*). Cinnamaldehyde is the main active chemical component found in cinnamon EO. The AgNP and cinnamaldehyde are engaged in the surface of pathogens which lead to the disruption of membrane and energy balance, and consequently the death of microorganisms [13]. Ghosh et al. [13] found a combination of AgNPs and cinnamaldehyde was synergistic against spore-forming *Bacillus cereus* and *Clostridium perfringens*, which supports the current study.

Generally, the major antibacterial and antifungal components in EOs are oxygenated terpenoids (such as phenolic terpenes, phenylpropanoids, and alcohols). However, they contain hydrocarbons (α-pinene, camphene, myrcene, α-terpinene, and *p*-cymene) showing low bioactivity when applied alone, but their effectiveness will increase in combination with the others [20,22]. For example, the hydrocarbon of *p*-cymene, found in Mediterranean formulation and cinnamon EO is known as a weaker antimicrobial component, but it can enhance the efficacy when combined with strong antimicrobial components (e.g., carvacrol). Because *p*-cymene is a substitutional impurity in the membrane having strong affinity binding to membranes resulting in the

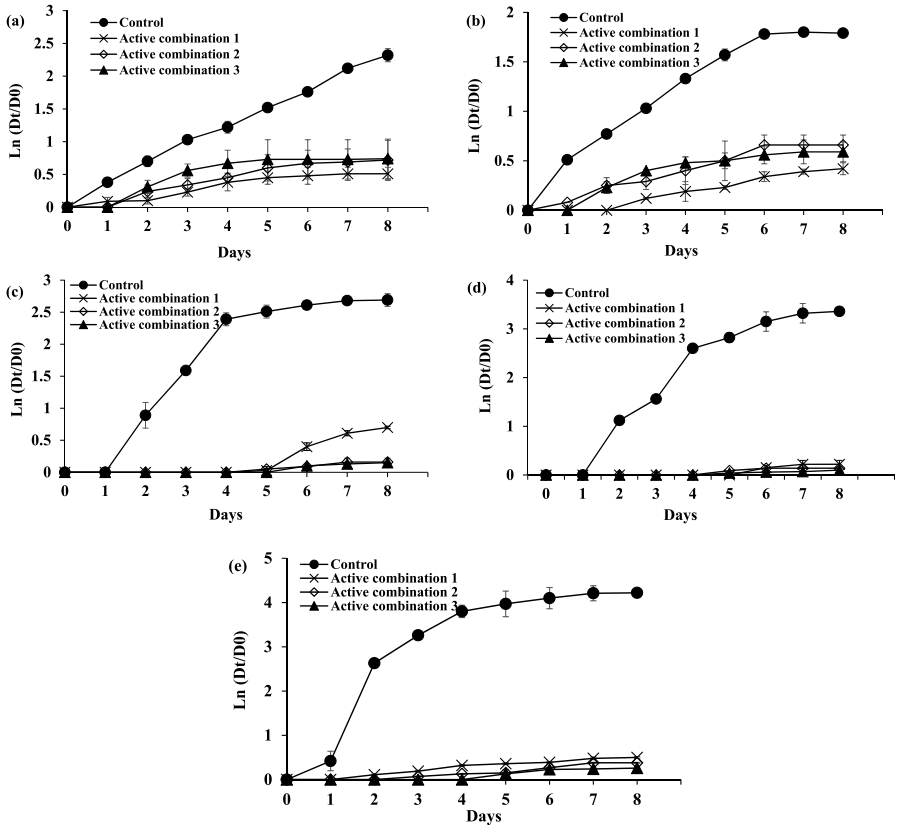

Fig. 1. Effect of active combinations 1, 2, 3 on the maximum colony diameter, Ln (D_t/D_0), of (a) *E. coli* O157:H7, (b) *S.* Typhimurium, (c) *A. niger*, (d) *P. chrysogenum*, and (e) *M. circinelloides* over time. The control sample did not contain active combinations. Values are means ± standard error.

decreased enthalpy and melting temperature of the membrane that facilitates carvacrol penetration easily into the cell [42]. The minor components in the EOs may have a significant influence on the major components to exert and cause synergistic effect. It has been hypothesized that the combined treatment of EOs and AgNPs could increase their applicability and EOs encapsulated by AgNPs may increase the physical stability and bioactivity of EOs thereby protecting the EOs from environmental influences [14].

Based on the FIC results, three active combinations (AGPPH+cinnamon EO, AGC 0.5+Mediterranean formulation+citrus EO and AGPP+cinnamon EO+Asian formulation+lavang EO) were selected for further tests as those combinations showed synergistic effect (FIC index) against all tested pathogenic bacteria (*E. coli* O157:H7, *S.* Typhimurium) and spoilage fungi (*A. niger*, *P. chrysogenum*, and *M. circinelloides*). The active combinations were denoted as active combination 1 (AGPPH: cinnamon EO (0.1 : 6)), active combination 2 (AGC 0.5: Mediterranean formulation: citrus EO (0.1 : 12: 6)), and active combination 3 (AGPP: cinnamon EO: Asian formulation: lavang EO (0.1 : 12: 6 : 6)). However, no study has been conducted in which the combination of two or more EOs with AgNPs is used to develop active combinations having synergistic effects. Hence, in the present study we verified the antibacterial and antifungal activities of the developed active combinations with synergistic effects from *in vitro* to *in situ* tests without contacting foods.

Table 5
Parameters of the modified Gompertz model for pathogenic bacterial and fungal species subjected to three active combinations containing essential oils and silver nanoparticles using vapor assay.

Active combinations	*E. coli* O157:H7			*S.* Typhimurium			*A. niger*			*P. chrysogenum*			*M. circinelloides*		
	A (cm)	$V_m d^{-1}$	λ (d)	A (cm)	$V_m d^{-1}$	λ (d)	A (cm)	$V_m d^{-1}$	λ (d)	A (cm)	$V_m d^{-1}$	λ (d)	A (cm)	$V_m d^{-1}$	λ (d)
Control*	2.4 ± 0.2^{b}	1.4 ± 0.04^{b}	0.7 ± 0.1^{a}	2.3 ± 0.04^{c}	1.3 ± 0.04^{c}	1 ± 0.2^{a}	3.1 ± 0.3^{b}	1.9 ± 0.04^{c}	1±0^{a}	3.2 ± 0.1^{b}	2.2 ± 0.04^{b}	1 ± 0^{a}	5.1 ± 0.1^{c}	3.3 ± 0.02^{b}	1±0^{a}
Active combination **1**	0.6 ± 0.1^{a}	0.3 ± 0.06^{a}	1±0^{a}	0.5 ± 0.01^{a}	0.2 ± 0.01^{a}	2±0^{b}	0.7 ± 0.1^{a}	0.2±0^{b}	4±0^{b}	0.23±0^{a}	0.08 ± 0.03^{a}	5±0^{b}	0.54 ± 0.06^{b}	0.3 ± 0.02^{a}	2±0^{ab}
Active combination **2**	0.7 ± 0.03^{a}	0.5 ± 0.1^{a}	1±0^{a}	0.5 ± 0.07^{a}	0.4 ± 0.1^{b}	1±0^{ab}	0.2±0^{a}	0.1 ± 0.01^{a}	4±0^{b}	0.19±0^{a}	0.07 ± 0.02^{a}	4±0^{b}	0.41 ± 0.02^{b}	0.2 ± 0.08^{a}	3±0^{bc}
Active combination **3**	0.7 ± 0.03^{a}	0.5 ± 0.02^{a}	1±0^{a}	0.7 ± 0.09^{b}	0.4 ± 0.1^{b}	1±0^{ab}	0.2±0^{a}	0.05±0^{a}	5±0^{b}	0.1±0^{a}	0.03 ± 0.02^{a}	4±0^{b}	0.16 ± 0.01^{a}	0.1±0^{a}	4±0^{c}

Control*, did not contain active combinations.
Values are means ± standard error. Within each column means with the same lowercase letter are not significantly different ($P \geq 0.05$).
A, Maximum colony diameter in cm during stationary phase; V_m, Maximum growth rate; λ, Lag time.

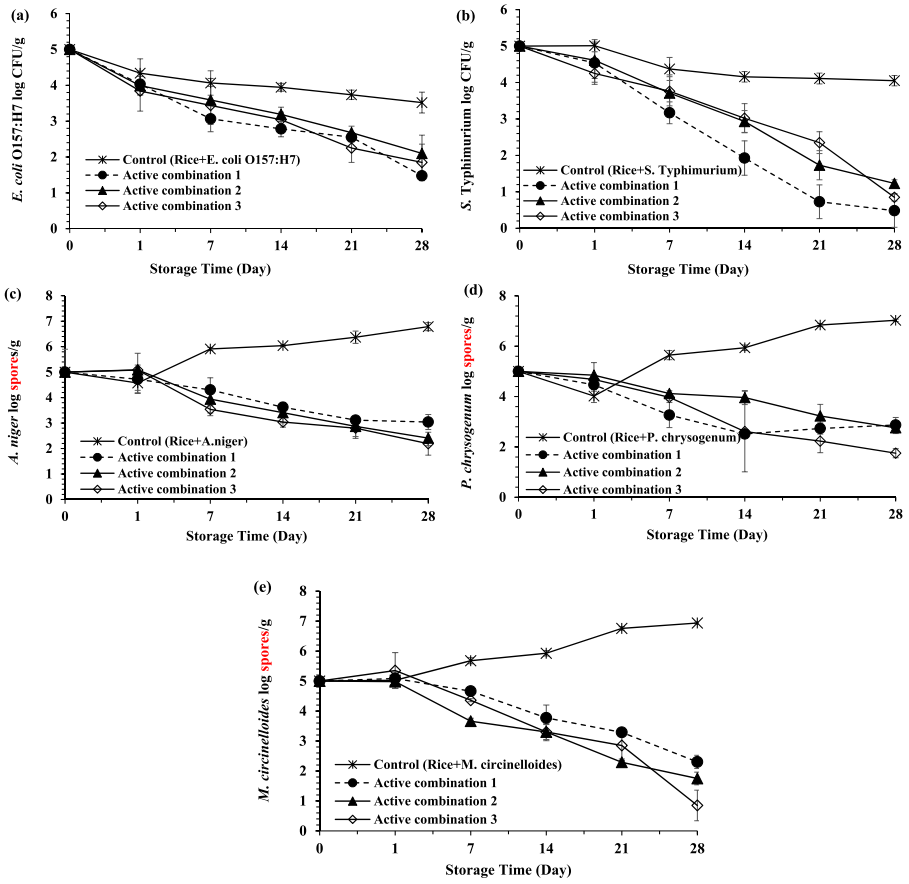

Fig. 2. Effect of active combinations (1, 2, and 3) in rice to control (a) *E. coli* O157:H7, (b) *S.* Typhimurium, (c) *A. niger*, (d) *P. chrysogenum*, and (e) *M. circinelloides*. The control sample did not contain antimicrobials/antifungals. Values are means ± standard error.

3.3. Vapor contact assay

The bacterial (*E. coli* O157:H7 and *S.* Typhimurium) and fungal (*A. niger*, *P. chrysogenum*, and *M. circinelloides*) colony diameter variation under the treatment of active combinations 1, 2, and 3 were evaluated and presented in Fig. 1. The modified Gompertz model was applied to compare the bacterial and fungal growth in the presence of active combinations 1, 2, and 3 through the parameters obtained from the model including the maximum colony diameter of the bacteria and fungi in stationary phase (A), the maximum exponential growth rate (V_m), and the lag time (λ) values [20,23,24].

It can be seen from Fig. 1 and Table 5, the active combination 1 shows the highest antimicrobial activity against *E. coli* O157:H7 having the maximum colony diameter or Ln (D_t/D_0) of 0.51, while that of the control is 2.32 on day 8. The lowest maximum growth rate (0.3/day) was observed in *E. coli* O157:H7 when treated with active combination 1, while the control sample showed the maximum growth rate of 1.4/day which was significantly different from that of active combination 1 ($P \leq 0.05$). Active combinations 2 and 3 showed the Ln (D_t/D_0) values of 0.72 and 0.74 against *E. coli* O157:H7, respectively. The three active combinations (1, 2, and 3) extended the lag phase of *E. coli* O157:H7 and *S.* Typhimurium from 1 to 2 days. The active combinations 1, 2, and 3 showed strong antimicrobial activity against *S.* Typhimurium, and the maximum colony diameter was 0.42, 0.66, and 0.59, respectively, while the control sample's Ln (D_t/D_0) was 1.79 ($P \leq 0.05$) (Fig. 1 b, Table 5).

The developed active combinations 1, 2, and 3 extended the lag phase of all tested fungal species *A. niger*, *P. chrysogenum*, and *M. circinelloides* to 2–5 days, while the control lag phase of tested fungi was 1–1.92 days ($P \leq 0.05$) (Fig. 1, Table 5). Active combination 3 was the most active against all three fungal species of *A. niger*, *P. chrysogenum*, and *M. circinelloides*. The maximum colony diameters (cm) of *A. niger*, *P. chrysogenum*, and *M. circinelloides* were 0.2, 0.1, and 0.16 when treated with active combination 3, respectively, while the corresponded maximum colony diameters for controls were significantly different 3.1, 3.2, and 5.1 cm at day 8 ($P \leq 0.05$) (Table 5). It was concluded that the active combinations 1, 2, and 3 limited the fungal growth, colony diameter, and extended the lag times. Similarly, Nikkhah et al. [22] studied the synergistic antifungal properties of the mixture of thyme, cinnamon, and rosemary EO against *P. expansum* and *Botrytis cinerea* by applying the modified Gompertz model to analyze the fungal growth profile. The authors found the mixture of thyme/cinnamon/rosemary EO was able to significantly reduce the fungal growth (*P. expansum* and *B. cinerea*) with an extended lag phase [22]. The oregano and thyme EO combinedly showed synergistic antifungal activity against *A. niger*, *A. flavus*, *A. parasiticus*, and *P. chrysogenum* reported by Hossain et al. [20]. Those authors introduced a modified Gompertz model to evaluate the antifungal efficacy of oregano/thyme EO which pronouncedly reduced the growth of *A. niger*, *A. parasiticus*, *A. flavus*, and *P. chrysogenum* and extended lag phase [20]. These findings provide valuable insights into how the active combinations exerted their antibacterial and antifungal activities by altering the growth kinetics of the tested pathogenic bacteria and fungi.

3.4. In situ antibacterial and antifungal efficiency of active combinations in rice

The vapor effect of active combinations 1, 2, and 3 containing essential oils and silver nanoparticles for controlling the pathogenic bacteria is presented in Fig. 2a and b and the result corresponding to fungi is shown in Fig. 2c, d, and 2e for a 28-day storage period in

Table 6
Antibacterial and antifungal efficiency of active combinations 1, 2, and 3 on tested bacteria and fungi in rice on day 28.

Active combinations	Bacterial and fungal growth in rice at 28 days (log CFU/g or spores/g)				
	E. coli O157:H7	*S.* Typhimurium	*A. niger*	*P. chrysogenum*	*M. circinelloides*
Control	3.52 ± 0.29^{bA}	4.05 ± 0.14^{cB}	6.79 ± 0.16^{cC}	7.03 ± 0.05^{cC}	6.93 ± 0.03^{cC}
Active combination 1	1.48 ± 0^{aB}	0.48 ± 0.1^{aA}	3.04 ± 0.29^{bD}	2.87 ± 0.29^{bD}	2.31 ± 0.22^{bC}
Active combination 2	2.9 ± 0.5^{aBC}	1.23 ± 0.1^{bA}	2.41 ± 0.3^{aC}	2.76 ± 0.23^{bC}	1.75 ± 0.21^{bAB}
Active combination 3	3.15 ± 0.5^{aB}	0.85 ± 0.1^{abA}	2.19 ± 0.45^{aB}	1.75 ± 0.18^{aAB}	0.85 ± 0.51^{aA}

Footnote: Values are means ± standard error. The same lowercase letter within each column either in the control or in the treated sample is not significantly different ($P > 0.05$). The same uppercase letters within each row are not significantly different ($P > 0.05$).

packaged rice. A 5 log CFU/g of bacteria or 5 log spores/g of fungi was inoculated in sterile rice samples and the control samples were not contain active combinations (contained only pathogens with rice).

The result showed that the active combination 1 had strong antimicrobial activity against *E. coli* O157:H7 and *S.* Typhimurium. A significant 3.52 and 4.52 log reduction of *E. coli* O157:H7 and *S.* Typhimurium was observed in rice when treated with active combination 1 as compared to control, respectively ($P \leq 0.05$). For *E. coli* O157:H7, the bacterial count was significantly ($P \leq 0.05$) reduced by 2.9 and 3.15 log CFU/g on 28th day of storage when treated with active combinations 2 and 3, while the bacterial count was 3.52 log CFU/g in the control. For *S.* Typhimurium, the bacterial count was 1.23 and 0.85 log CFU/g when treated with active combinations 2 and 3, respectively, while the control sample showed 4.05 log CFU/g after a 28-day storage period ($P \leq 0.05$). However, it was observed that *S.* Typhimurium was more sensitive to all three active combinations compared to *E. coli* O157:H7 (Fig. 2 a,b; Table 6).

The active combinations 1, 2, and 3 were significantly ($P \leq 0.05$) reduced *A. niger*, *P. chrysogenum*, and *M. circinelloides* count in packaged rice on 28th day (Fig. 2 c,d,e). Active combination 3 showed the highest antifungal activities against all tested fungi as compared to the control. At 28 days of the storage period, the three fungal (*A. niger*, *P. chrysogenum*, and *M. circinelloides*) counts significantly reduced by 2.19, 1.75, and 0.85 spores/g when treated with active combination 3, respectively, while the corresponded fungal counts in controls were 6.79, 7.03 and 6.93 spores/g ($P \leq 0.05$) (Table 6).

For *A. niger*, the fungal count was 3.04 and 2.41 spores/g in the sample treated with active combinations 1 and 2 ($P \leq 0.05$), respectively. The active combinations 1 and 2 reduced *P. chrysogenum* count by 2.87 and 2.76 spores/g ($P > 0.05$), respectively. These values for *M. circinelloides were* 2.31 and 1.75 spores/g in rice stored for 28 days, respectively. *M. circinelloides* was more sensitive to active combinations 1, 2, and 3, followed by *P. chrysogenum* and *A. niger*. Overall, the vapor of combined EOs and AgNPs can significantly ($P \leq 0.05$) reduce the pathogenic bacteria and spoilage fungi during the storage of rice. Likewise, low-density polyethylene (LDPE) film containing bimetallic nanoparticles (4% Ag–Au NPs) with *cinnamon* EO showed strong antibacterial activities against *S.* Typhimurium, *L. monocytogenes*, and *Campylobacter jejuni* at during 21 days meat storage at 4 °C. It was also observed that combined Ag–Au/cinnamon EO was capable of a 100% reduction of *S.* Typhimurium and *C. jejuni* from meat during the storage [43]. Another study was conducted by Dehkordi et al. [44] to evaluate the antibacterial properties of the AgNPs and eugenol, alone and in combination, against *S. aureus* and *S.* Typhimurium in meat and milk. The authors found combine (AgNPs/eugenol) treatment achieved 6 log reduction of *S.* Typhimurium and *S. aureus* from meat and milk samples within 3 h and 24 h, respectively, while it took a long time to reach a 6-log reduction of those bacteria when a single antimicrobial agent was applied [44].

4. Conclusion

Our study highlights the antimicrobial and antifungal efficiency of EOs and AgNPs used alone or in combination to control pathogenic bacteria (*E. coli* O157:H7 and *S.* Typhimurium) and spoilage fungi (*A. niger*, *P. chrysogenum*, and *M. circinelloides*) during the storage of rice. The spherical shaped AgNPs (AGPP, AGPPH, AGC 1, and AGC 0.5) and EOs (cinnamon EO, Mediterranean formulation, citrus EO) showed strong to moderate antibacterial and antifungal efficacy in MIC assays. Three active combinations 1, 2 and 3 were developed using Checkerboard analyses which showed synergistic activity (FIC <1.0) against all tested bacteria and fungi, and active combinations were (1) AGPPH+cinnamon EOs (active combination 1) (2) AGC 0.5+Mediterranean formulation+citrus EO (active combination 2) and (3) AGPP+cinnamon EO+Asian formulation+lavang EO (active combination 3). A modified Gompertz model was used to obtain more information on the growth profile of bacteria and fungi when treated with active combinations 1, 2, and 3 in the vapor state. *In vitro* vapor effect of active combinations 1, 2 and 3 was significantly reduced the growth rate of the tested bacteria and fungi by prolonging their lag phase as compared to the control sample (without active compounds). The more challenging *in situ* tests were performed in rice to evaluate the antimicrobial and antifungal effects of active combinations under more realistic conditions. The antimicrobial and antifungal effect of active combinations 1, 2, and 3 significantly decreased the count of *E. coli* O157:H7, *S.* Typhimurium, *A. niger*, *P. chrysogenum*, and *M. circinelloides* in rice samples during a 28-day storage period. Silver nanoparticles and essential oil combinations are a potential alternative and natural way to control pathogens and spoilage fungi in food products during storage, providing increased shelf life and preventing postharvest losses.

CRediT authorship contribution statement

Tofa Begum: Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Peter A. Follett:** Conceptualization, Methodology, Software, Supervision, Writing – review & editing. **Jumana Mahmud:** Data curation. **Lana Moskovchenko:** Methodology. **Stephane Salmieri:** Methodology. **Zahra Allahdad:** Methodology. **Monique Lacroix:** Conceptualization, Methodology, Software, Supervision.

Declaration of competing interest

All authors state that there is no conflict of interest.

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