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Effect of Lemongrass Oil Vapours and Negative Air Ions on Food Spoiling Microbes

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Abstract—*The aim of this study was to investigate the antimicrobial potential of lemongrass oil vapours (LGOV) and negative air ions (NAI) alone and in combination against food spoiling microbes such as Saccharomyces cerevisiae, Listeria monocytogenes and Pichia anomala, using a kill time assay in a specially designed set-up. The exposure to NAI for a period of 8h showed 28.15%, 49.91%, 36.59% reduction in viability for S. cerevisiae, L. monocytogenes and P. anomala, respectively. Whereas a significant reduction in viability was observed due to LGOV (72%, 77.37% and 49.24%) against S. cerevisiae, L. monocytogenes and P. anomala, respectively. The combinational effect of NAI and LGOV against S. cerevisiae, L. monocytogenes and P. anomala exhibited an increase in viability reduction to 81%, 80.4% and 63.13%, respectively, after 8h. The kill time assays has clearly highlighted that the combination of LGOV with NAI enhanced the efficacy over NAI or LGOV alone. The present study offers a novel approach to integrate NAI and LGOV for preventing microbial deterioration of the food products thereby increasing their shelf lives.*

Keywords*: Lemongrass oil, NAI, S. cerevisiae, L. monocytogenes and P. anomala.*

1. INTRODUCTION

Rural areas provide a suitable location for installation of agro-based industries. However, deterioration of foodingredients is a bigchallenge for food industries. In order to deal with theseissues, synthetic preservatives are widely used to control microbial growth thus increasing the shelf lives of food materials. However, due to increasing health problems associated with chemicals, immediate attentionis required to substitute these with natural preservatives which doesn't cause any unwanted harm to the food materials.

The use of plant based essential oilshave been gaining importance due to presence of naturally occurring components for preservation of food products[1, 2]. An essential oil isobtained generally by distillation process andcontains the volatile aroma of the source plants. It was observed that their effect in food is only achieved with higher doses as compared to the minimum inhibitory concentration (MIC) in nutrient media [3, 4], causing organoleptic impact. However, some recent studies [5, 6, 7, 8] have found that the vapours of essential oils can be a good option to overcome these problems. It was reported that the vapours of essential oils not only prevents the unwanted (organoleptic effect) effect due to high doses but are also efficient in controlling the microbial load at very low concentrations.

Studies have also highlighted the role of negative air ions (NAI) for reducing the microbes responsible for decaying food materials [9, 10]. NAI are formed by the attachment of free electrons at N_2, CO_2, O_2 etc. NAI generators providesa sustainable source of NAI, which possess considerableantimicrobialpotential[8] Employing NAI with essential oils not only reduces the requirement of high doses but can also produce remarkable enhancement in preventing the unwanted microbes from edibles. Being a simple technology, it shall be accessible for rural entrepreneur. Therefore, the aim of this study was to investigate the antimicrobial potential of LGOV and NAI against food spoiling microbes such as *S. cerevisiae, L.monocytogenes*and *P.anomala*.

2. MATERIALS AND METHODS

2.1 Essential oil

The essential oil (Lemongrass oil) was procured from Natural Aromatics Private Limited, New Delhi (India). Growth media was purchased from HimediaPvt. Ltd. (India).

2.2 Microbial strains

Bacterial and yeast strains (*L.monocytogenes*, *P.anomala*and *S. cerevisiae*) were collected from the microbial culture collection of food microbiology laboratory, University of Bologna, Italy.

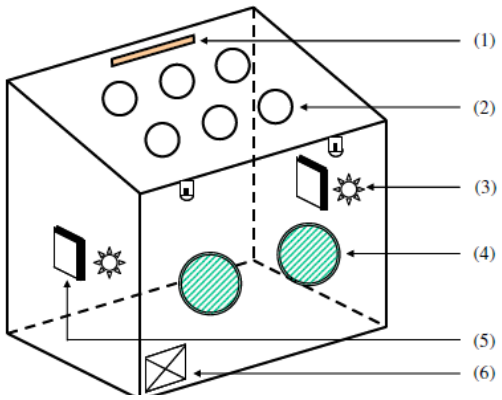
2.3 Preparation of microbial culture

The bacterial strain was grown in Muller Hinton broth (MHB) medium at 30 °C, 180 rpm for 24 h and the yeast strains were grown in Potatoe dextrose broth (PDB) medium at 28 °C, 160

rpm for 24 h. Cells were harvested by centrifugation, suspended in sterile distilled water and plated on MHA/ PDA medium for doing NAI and LGOV experiments. The results so obtained were analysed and a comparative study of the effect of NAI and LGOV on selected yeast and bacterial strains was performed.

2.4 Exposure regimes

A set-up was used [8] to study the antimicrobial efficacy of NAI alone, LGOV alone and combination of NAI and LGOV. A compact chamber made up of acrylic material (size 50 cm×50 cm; W×L) was used for this purpose (Fig. 1). The height of the chamber was 50 cm on the back side and 25 cm at the front side. The total volume of the chamber was 0.09375 m3 (93.75 l). The front side of the chamber had gloves through which the things inside the chamber could be handled without opening the chamber. Prior to exposure, the chamber was cleaned with ethanol and UV sterilized. Essential oil vapour (EOV) generators (evaporation rate=0.5 ml/h, Khera brothers Pvt. Ltd., New Delhi) and two NAI generators were fixed in this chamber. A negative air ion generator which contains several needle shaped electrodes to provide the negative charge to the air ions, was used. The NAI generators were positioned such that a continuous concentration of about 10^6 NAI/ml (monitored by an Air Ion Counter, Alpha Lab, Inc.), was maintained [9]. For EOV/NAI exposure, appropriate serial dilution of the culture (to obtain 100-300 cfu) was plated on MHA/PDA plates. The plates were opened inside the chamber and fixed at different locations with the help of double-sided tape. After a particular time period (0.5, 1, 2, 4, 6 and 8h) the plates were detached, closed and incubated at 30 °C for bacterial strain and 28 °C for yeast strains for 20-24 h.



(Source: Tyagi et al., 2012)

Fig. 1: Exposure regimes for kill time assay. (A) Line diagram of the experimental set up showing (1) UV lamp, (2) Petri-plates, (3) Essential oil vapour (EOV) generator, (4) Gloved openings, (5) Negative air ion (NAI) generator, (6) Electrical sockets.

For studying the effect of NAI, only NAI generator was switched on and it was ensured that the chamber did not contain any residual vapour. For investigating the effect LGOV alone, only EOV generator was switched on. For studying the effect of combination of LGOV with NAI, both EOV and NAI generator were switched on. The control plates were kept closed under similar conditions during the exposure period. The results were represented as percentage reduction in viability in treated plates in relation to the untreated control plates.

3. RESULTS AND DISCUSSION

The antimicrobial potential of the active agents (LGOV, NAI, LGOV+NAI) against *S. cerevisiae, L. monocytogenes*and *P. anomala* were evaluated using the kill time assays. The assays were done by exposing the inoculated plates (100-300 cfu) to NAI alone, LGOV alone, and combination of LGOV and NAI in a closed airtight chamber for 8 h. The effect of NAI exposure on *S. cerevisiae, L. monocytogenes and P. anomala* is shown in Fig. 2. After 8 h NAI exposure, the reduction in viability was recorded as 28.15%, 49.91% and 36.59%, respectively. [9], has achieved comparable results (33% and 42%) within 4 h exposure against *E.coli* and *P. fluorescens strains*, respectively. It was also observed that 3h exposure of NAI can reduce the cfu (103–53 CFU/plate) of *S. enteritidis*[11]. Further assays were conducted to assess the antimicrobial efficacy of the LGOV by exposing the inoculated plates (100-300 cfu) to LGOV in a closed air tight chamber. The LGOV exposure revealed substantial reduction in viability (72%, 77.37% and 49.24%) of *S. cerevisiae, L. monocytogenes*and *P. anomala*, respectively, after 8h of exposure (Fig. 3). [12], reported superior antimicrobial activity (100%) from LGOV against *C. albicans* in 4 hours. Lopez et al. (2007) studied the effect of essential oils vapours (Cinnamon, Oregano, Thyme) against bacteria (gram positive & gram negative), molds and yeast and concluded that the EO vapours are effective antimicrobial agent. The results suggested that MICs were generally lower for oregano than for thyme and cinnamon essential oils. The combined exposure of both NAI and LGOVagainst microbes has established the higher reduction potential in the present study. The exposure assay (NAI+LGOV) revealed 81%, 80.4% and 63.13% of viability reduction against *S. cerevisiae, L. monocytogenes*and *P. anomala*, respectively, after 8h (Fig. 4). The combinational studies using NAI+EOV were also undertaken i.e. Tyagi et al, 2012; where the integration effect of NAI and LGOV on *E.coli* was found to be more effective (100% viability reduction) than observed with the present study. The results of the kill time assays in the present study have indicated that the combination of NAI with LGOV has a greater antimicrobial effect (63-81% reduction in viability of selected microbes) than NAI (28-49 %) or LGOV alone (49-77%) within 8 h exposure.

The medicinal importance of lemon grass oil vapours have also been reported in several studies i.e. lemongrass oil was found to be an effective control agent in gaseous phase for respiratory tract pathogens [14]. Lemongrass oil was also observed to decrease the serum cholesterol concentration in Hypercholesteraemic subjects [15]. The chemical analysis revealed that lemon grass essential oil contains oxygenated

monoterpenes (78.2%); α-citral or geranial (36.2%) and β-citral or neral (26.5%), monoterpene hydrocarbons (7.9%) and sesquiterpene hydrocarbons (3.8%) [12]. Monoterpene hydrocarbons play an important role with oxygenated monoterpenes in vapour phase. The potent activity of LGOV could be due to better penetration and solubilisation ability of monoterpenes in gaseous form. The antimicrobial activity can further be enhanced by integration with NAI for long term preservation of edibles. High volatility of LGOV makes it an excellent antimicrobial agent in vapour phase which can be easily integrated with NAI for enhanced efficacy. The present study highlighted a simple, safe and eco-friendly approach to use "LGOV+NAI" for protecting the food products against food spoiling microbes thus strengthens the knowledge and importance of green technology which further helps in its adoption and usage.

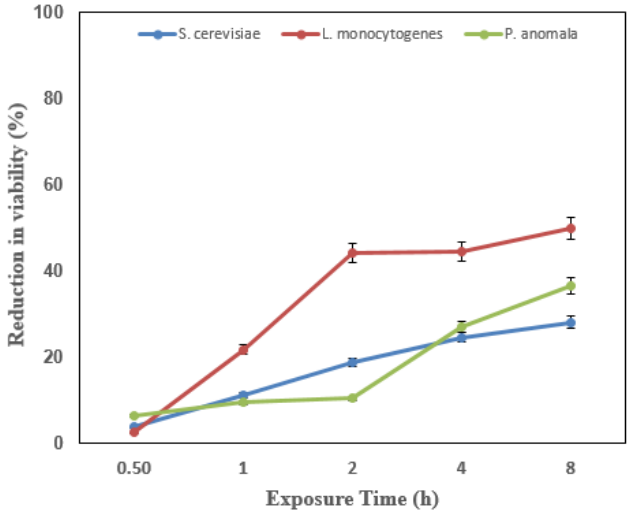


Fig. 2: Effect of NAI exposure on *S. cerevisiae, L. monocytogenes, P. anomala*

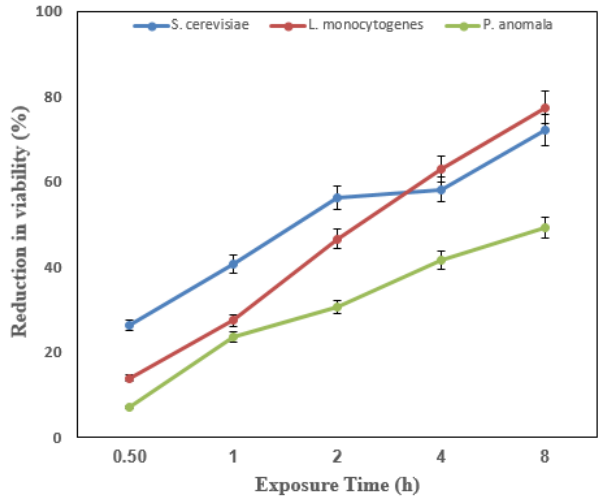


Fig. 3: Antimicrobial potential of LGO in vapour phase against *S. cerevisiae, L. monocytogenes, P. anomala*

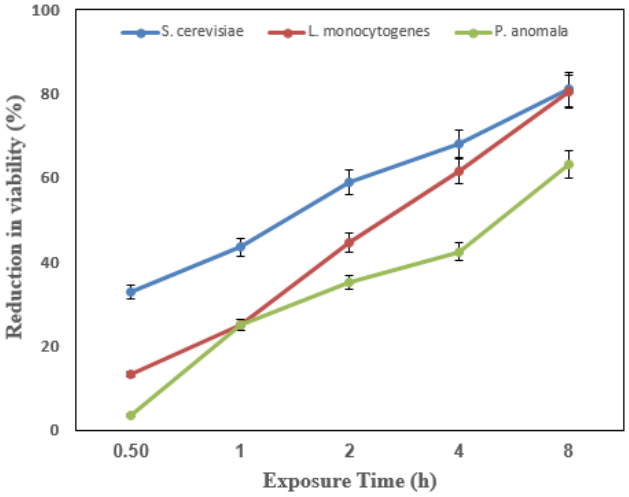


Fig. 4: The combinational exposure of NAI+LGO vapours on *S. cerevisiae, L. monocytogenes, P. anomala*

4. CONCLUSION

The results obtained in the present study clearly suggested that lemon grass oil is highly efficient in vapour phase for controlling the growth of food spoiling microbes. The efficacy of LGOV can further be enhanced by integrating it with NAI which showed an increased viability reduction than LGOV or NAI alone against *S. cerevisiae, L. monocytogenes and P. anomala*. Further, the testing of other harmful food microbes would reinforce this system asantimicrobial/ preservativeof food products especially to rural sectors which lacks the facilities of disinfection and long term preservation of edibles.

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