



RESEARCH ARTICLE

In vitro antifungal activities of *Cymbopogon citratus*, *Cymbopogon martini*, *Pogestemon cablin* and *Curcuma longa* essential oils against spoilage fungi isolated from tomatoes

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Abstract

Tomatoes (*Solanum lycopersicum* L.) have high moisture content (93–95 %), making them more vulnerable to various types of spoilage fungi. Numerous medicinal plants' essential oils are known to have antifungal properties that combat these spoilage fungi. Therefore, this study aimed to investigate the potential antifungal activity of essential oils from some indigenous plants in Bangladesh to prevent the fungal attack on tomatoes. Using potato dextrose agar medium, 4 species of spoilage fungi from tomatoes were identified. ITS (Internal transcribed spacer) region-specific sequencing was used to identify the fungal species as *Aspergillus welwitschiae*, *Aspergillus tamari*, *Penicillium citrinum* and *Rhizopus arrhizus*. Assessment on antifungal activity was conducted for four essential oils extracted from plants: turmeric leaf (*Curcuma longa*), patchouli (*Pogestemon cablin*), palmarosa (*Cymbopogon martini*) and lemongrass (*Cymbopogon citratus*). The GC-MS method was used to analyze these essential oils to ascertain their chemical content. A pathogenicity test determined the severity and % of rot on tomatoes. *A. welwitschiae* caused the most severe spoilage (80 %) after 21 days, while *R. arrhizus* damaged 26 % of the tomatoes within 5 days. The minimal fungicidal concentration (MFC) and minimal inhibitory concentration (MIC) of the essential oils were determined after analysis. Lemongrass oil fully suppressed the growth of all the isolates at a concentration of 12 µl/ml. Finally, the essential oils of palmarosa and lemongrass may be used as a preventative measure against tomato fungal rot.

Keywords

antifungal activity; essential oils; *Solanum lycopersicum*; spoilage fungi

Introduction

From the Solanaceae family, the tomato (*Solanum lycopersicum* L.) is one of the most widely consumed fruits in the world, including Bangladesh (1). Tomato plants are grown in practically all the fields and backyard gardens in this area because of their adaptability to a variety of soil types and climates (1). Tomatoes are consumed mainly in fresh and processed forms (2) and contain proteins, fats, fibers, potassium, carbohydrates, various vita-

mins and lycopene (3). Lycopene increases the skin's protection ability against ultraviolet rays, prevents prostate cancer and decreases the cardiovascular risk associated with type 2 diabetes and urinary tract infections (4). In Bangladesh, about 445 thousand MT of tomatoes were produced during the years 2020-21 (5). However, one-fourth of the tomatoes produced become bruised and unusable (6) due to environmental changes, pests, low rainfall and fungal attacks, among other reasons (7). Post-harvest diseases significantly contribute to tomato loss, resulting in several-fold more losses compared to other fresh fruits during transportation from the field to consumers (8, 9).

The significant amount of water in tomatoes makes them more susceptible to spoilage by microorganisms (4). In fruits, fungi outgrow bacteria as they prefer to grow at low pH (10). Fungi are the most significant and prevalent pathogens capable of infecting a wide variety of host plants, causing destruction and economic loss in tomatoes in the field, during storage or transportation (11-13). Microbial spoilage in tomato fruits can occur due to several fungal genera, including, *Alternaria*, *Fusarium*, *Penicillium*, *Aspergillus*, *Geotrichum*, *Phytophthora*, and *Botrytis*, among others (11, 14). Fungi produce mycotoxins that can cause mycotoxicosis in humans after ingestion or inhalation. Pathogens affecting tomatoes can lead to various diseases, such as meningitis, gastroenteritis and diarrhoea, when consumed raw (4). Sour rot, rhizopus rot, buckeye rot and black mould rot are the major post-harvest diseases caused by fungi (9, 11).

Chemical, biological and physical treatments are widely applied to prevent fungal growth, mycotoxin biosynthesis and food contamination (15). A study shows that a moderate number of tomatoes in many Bangladeshi marketplaces are tainted with artificial ripening agents such as CaC₂ and ethylene (16). Formalin is used to preserve fruits because it kills bacteria and other microorganisms that can cause spoilage. Dithane is a broad-spectrum fungicide that controls various fungal diseases in fruits, vegetables and field crops. However, these chemicals are highly toxic and pose health hazards (16). Furthermore, microbial resistance, toxicity to living organisms and long-term persistence in the environment are drawbacks to the use of synthetic chemicals (17). Nowadays, researchers have turned their attention to plant-derived fungicides as alternatives to synthetic fungicides (18-20). Natural antimicrobial substances are biodegradable, have a broad spectrum of activity and are less likely to cause adverse consequences (15). Essential oils are among the most effective sources of natural antimicrobials that exhibit antimicrobial activity against plant and human pathogenic microbes (21, 22). The most frequently used essential oils as natural food preservatives include cinnamon, clove, lemongrass, oregano, thyme, nutmeg and basil (21). Essential oils of *Cymbopogon citratus*, *Cymbopogon martini*, *Pogestemon cablin* and *Curcuma longa* are known to contain several active compounds such as citral, neryl, geraniol, neryl acetate, neryl methyl ether, α guaine, p-cymenol and p-cymene. Some of these compounds are known to

possess potent antifungal activity (23-25). In Bangladesh, the fungi causing tomato spoilage have been reported (26), but the antifungal activity of plant essential oils against tomato-derived spoilage fungi has not yet been investigated.

In this study, we investigated the antifungal properties of essential oils extracted from *C. citratus*, *C. martini*, *P. cablin* and *C. longa* against spoilage fungi derived from tomatoes. Five spoilage fungi were isolated from rotten tomatoes and pure culture was obtained on potato dextrose agar media. The selection of target spoilage fungi was based on their pathogenicity test results following Koch's postulate. MIC and MFC tests of *C. citratus* and *C. martini* essential oils on susceptible fungi showed superior inhibitory activities, suggesting their potential use as organic fungicides. Further analysis of these essential oils using GC-MS supports this hypothesis.

Materials and Methods

Sample collection

At the beginning of the summer season (June 2019), 24 fresh tomato samples (almost identical sizes) were collected in sterile polythene bags from the local market of Baluchara Bazar, Chattogram, Bangladesh. These samples were immediately transported to the Industrial Microbiology Research Division, Bangladesh Council of Scientific and Industrial Research (BCSIR), Chattogram Laboratories. Upon arrival, the tomatoes were thoroughly washed with distilled water and then air-dried. Subsequently, they were stored in several sterile paper boxes at room temperature and allowed to spoil. The spoiled tomatoes were then utilized as a study sample for the present work.

Isolation of fungi

After 21 days, visible sections of fungal mycelial from the contaminated parts of the rotten tomatoes were inoculated onto PDA media (HiMedia Laboratories, India) using a sterile inoculating loop under aseptic conditions. The plates were then incubated for 7 days at 25 °C and the fungal isolates were sub-cultured and re-incubated repeatedly. The single spore method was employed for the purification of the isolates.

Fungal identification

The morphological features of fungi, including color, shape, size and hyphae were analyzed. Species-level identification was conducted through ITS barcode region sequencing. DNA extraction and sequencing of the isolated fungal strains were performed by 1st Base Laboratories, Malaysia. ITS4 and ITS5 primers were used to amplify the internal transcribed spacer region (the 5.8S gene) in filamentous fungi (Table 1) (27). The obtained sequences

Table 1. Primers used for ITS region sequencing (28).

| Category | Primer name | Position | Primer Sequence (5' -3') |
|---------------|-------------|-----------|--------------------------|
| SSU (Forward) | ITS5 | 1737-1758 | GGAAGTAAAAGTCGTAACAAGG |
| LSU (Reverse) | ITS4 | 2390-2409 | TCCTCCGCTTATTGATATGC |

were uploaded to the NCBI nucleotide database.

Pathogenicity test

We employed Koch's postulates to confirm that a specific isolate was the causative agent of the observed spoilage. Following the protocol outlined by Fawole and Oso (28), pure cultures of spoilage fungi were cultivated on PDA media after being isolated from diseased tomatoes. A batch of healthy tomatoes was then reintroduced to the isolated fungus. Upon re-isolation, the isolates were shown to be identical to the initial causal agent (28). In the beginning, the surface of healthy tomatoes was sterilized for 1 min with 70 % ethanol and subsequently washed with distilled water 5 times (29). Five groups of healthy tomatoes were segregated into 5 sterile boxes for the experiment. The weight of the tomatoes was determined using the following formula:

$$\text{Expected weight} = \text{Total weight} - \text{the weight of the box}$$

A 4 mm deep wound was made in healthy tomato fruits using a sterile 5 mm cork borer and the bored tissues were removed. A 5 mm diameter disc from fresh fungal cultures was then placed inside the bored hole on the tomatoes. The wounds were covered with candle wax following the method of Fawole and Oso (28). A group of 5 tomatoes was kept as the control without any wounds or inoculation. The boxes were sealed with parafilm and incubated at 28 °C for 21 days. Samples were checked after 5, 7, 14 and 21 days and the boxes were reweighed. Water released from rotten tomatoes was frequently discarded. The severity of rots caused by these fungi was determined using the process reported (30).

$$\text{Severity} = W - w \times 100\% / W$$

Where, W = Initial weight of the healthy tomato; w = Final weight of the rotten tomato.

Essential oil extraction

Four aromatic plants, namely lemongrass, palmarosa, patchouli and turmeric (Table 2), were cultivated on the premises of BCSIR Chattogram Laboratories, Bangladesh, located at 22°24'35.4" N and 91°49'00.6" E in the South-Eastern part of the country. These plants were selected based on literature studies regarding their antifungal properties, ease of harvesting, prolific growth and accessibility in this region. Lemongrass, palmarosa and patchouli were planted in the first week of January and mature leaves were harvested in the first week of July, while turmeric was planted in the first week of May, with its leaves harvested in the first week of March. Fresh mature leaves of lemongrass, palmarosa and patchouli were used for essential oil extraction. In contrast, turmeric leaves were collected when mature and fresh, then air-dried before extraction. The extraction of oils was carried out using a Clevenger glass apparatus through hydro-distillation. Anhydrous sodium sulphate was used to remove excess water from the extracted oils. The oil % of lemongrass, palmarosa, patchouli and turmeric leaves were 0.5 (V/FW), 1.0

(V/FW), 0.27 (V/FW) and 2.0 (V/DW) respectively. Essential oil samples were preserved at 4 °C until further analysis. The identification of the 4 aromatic plants - lemongrass, palmarosa, patchouli and turmeric was conducted by Nemaï Chandra Nandi, Medicinal and Aromatic Plant Research Division, BCSIR Chattogram and deposited at the Industrial Botany Research Division's Herbarium, BCSIR Chattogram, with voucher numbers 1517, 1518, 980 and

Table 2. Essential oils used for the study.

| S. No. | Essential Oil | Scientific Name | Bangladeshi Local Name |
|--------|---------------|----------------------------|------------------------|
| 1. | Lemongrass | <i>Cymbopogon citratus</i> | Lebugandhi ghas |
| 2. | Palmarosa | <i>Cymbopogon martinii</i> | Palmrosa ghas |
| 3. | Patchouli | <i>Pogostemon cablin</i> | Bangali juilata |
| 4. | Turmeric leaf | <i>Curcuma longa</i> | Halud, Haldi |

748(b) respectively.

GC-MS analysis of essential oils

The essential oils were analyzed using a high-end single quadrupole GC-MS-QP2020 machine (Shimadzu, Japan). Briefly, 2 mL oil samples were filtered using a Millipore filter with a pore size of 0.22 µm and then 4 µL of the filtered sample was injected into the GC-MS system with a split ratio of 80.0. The analysis was performed using an SH-Rxi-5Sil MS capillary column, which is composed of 5 % diphenyl and 95 % dimethylpolysiloxane, with dimensions of 30 m (length) × 0.25 mm (inner diameter) and a film thickness of 0.025 µm. Helium gas served as a carrier with a flow rate of 0.07 mL/min. The injection temperature was maintained at 230 °C and electron impact ionization (EI) was recorded over the mass range of 30 to 300 m/z. The separated peaks were compared with spectra in the NIST08, LIB program library databases for identification.

Antifungal activities of essential oils

The antifungal activity of the essential oils was assessed using the agar medium assay (31). Five specific concentrations (3, 6, 9, 12 and 15 µl/ml) were prepared by adding the appropriate amount of essential oils and 0.5 % (v/v) Tween 80 to PDA media. The media was solidified at room temperature (25 ± 3 °C) for approximately 1 h. Agar discs containing mycelia (5 mm in diameter) were obtained from actively growing cultures of seven-day-old pure cultures with a sterile cork borer and aseptically placed at the centre of the petri plates. Control plates were inoculated with fungal strains without the essential oils. The plates were sealed with parafilm and incubated at 28 °C. The diameter of fungal colonies was measured after 5 and 7 days of incubation. The % of mycelial growth inhibition caused by the essential oils was calculated using the following formula (32).

$$\text{Inhibition of mycelial growth (\%)} = dc - dt / dc \times 100$$

Where, *dc* is the mean diameter of the colony in the control sample and *dt* is the mean diameter of the colony in

the treated sample.

MIC and MFC

The lowest concentration of essential oils at which no visible growth occurred was defined as the Minimum Inhibitory Concentration (MIC). To assess the biocidal activity of essential oils on the test fungi, the Minimum Fungicidal Concentration (MFC) of the oils was determined. From plates treated with oils, fungal discs that were inhibited were re-inoculated onto newly prepared PDA media and their growth was observed after incubating the plates at 28 °C for three days. The lowest concentration of the oils at which no growth occurred after sub-culturing was considered the MFC (33).

Statistical analysis

The experiments were conducted three times for each sample and each dose. A comparison of the mycelium with the controls was used to express the results. Variance analysis (one-way ANOVA) was employed to assess the significance of the results. A significance level of $p < 0.05$ was considered significant and the data were documented as mean \pm standard error (SE).

Results and discussion

Morphological and Molecular Identification of fungi

From the collected tomatoes, we isolated four harmful fungi. Based on their macroscopic morphological traits, the fungal species were identified and verified by mycological sources. All the strains were filamentous fungi (Fig. 1). Using the ITS region-specific sequencing technique, the isolated fungal strains were identified as: *A. welwitschiae* imrd 1 (F1B), *A. tamari* imrd 3 (F3BR), *Rhizopus arrhizus* imrd 5 (F4BW) and *Penicillium citrinum* imrd 4 (F5G) (Table 3).

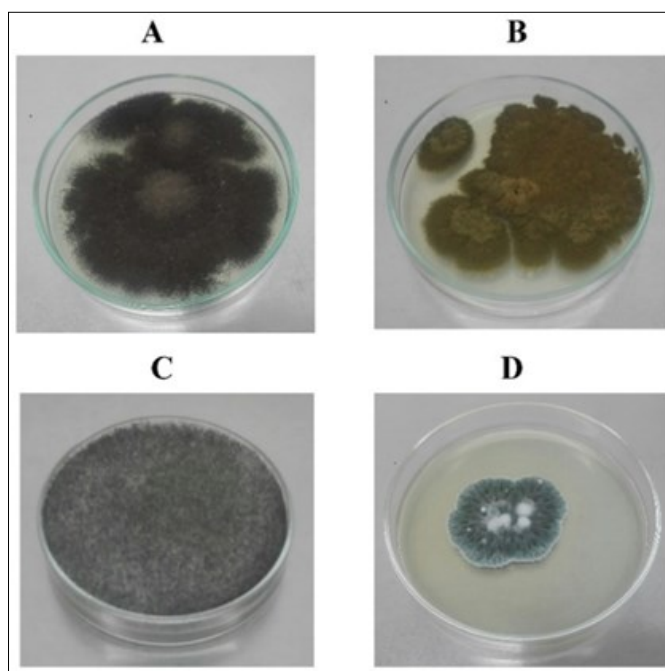


Fig. 1. Isolated pathogenic fungus on potato dextrose agar. (A) *A. welwitschiae* imrd 1 (F1B), (B) *A. tamari* imrd 3 (F3BR), (C) *Rhizopus arrhizus* imrd 5 (F4BW), and (D) *Penicillium citrinum* imrd 4 (F5G).

Table 3. Molecular identification of isolates.

| S. No. | Isolate id | Species Identified | Accession Number |
|--------|------------|--|------------------|
| 1. | F1B | <i>Aspergillus welwitschiae</i> imrd 1 | MW332490 |
| 2. | F3BR | <i>Aspergillus tamari</i> imrd 3 | MW332492 |
| 3. | F4BW | <i>Rhizopus arrhizus</i> imrd 5 | MW332494 |
| 4. | F5G | <i>Penicillium citrinum</i> imrd 4 | MW332493 |

Pathogenicity test

The severity of spoilage (Fig. 2) varied across the different fungal species and days of inoculation. The results of the pathogenicity tests supported Koch's postulate, demonstrating that the fungi inoculated into the healthy tomatoes and those re-isolated from them exhibited the same features, indicating that these fungi were responsible for tomato spoilage (34). In comparison to the tomato batch that was inoculated, the control group of tomatoes exhibited delayed signs of rotting. *R. arrhizus* (F4BW) was observed to cause 26 % of tomato spoilage within 5 days, whereas the most severe rot (80 %) was attributed to *A. welwitschiae* (F1B) within 21 days. *A. tamari* (F3BR) and *P. citrinum* (F5G) were found to be less pathogenic, causing 29 % and 27.9 % of spoilage in 21 days respectively. No symptoms of spoilage were observed in the control fruits. The present work supports the previous study that reveals *Penicillium* sp. and *A. niger* as spoilage fungi of tomatoes from Benin City in southern Nigeria (35). *Rhizopus* spp. is also found to be responsible for spoilage in tomatoes in the markets of Maiduguri in North-Eastern Nigeria (36).

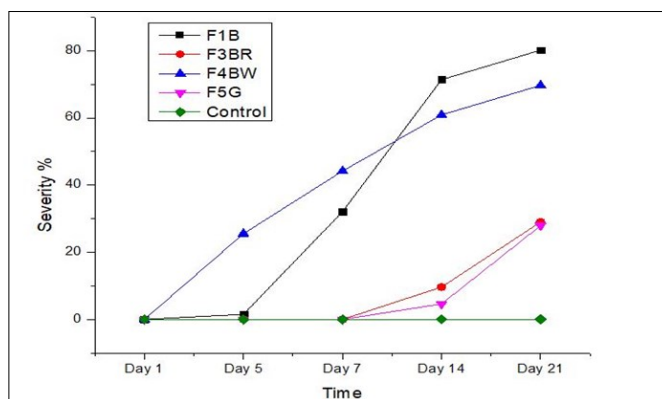


Fig. 2. Severity of spoilage caused by isolated fungi from day 1 - 21.

GC-MS analysis of essential oils

GC-MS analysis revealed a variety of components in the 4 different essential oils. The major components of palmarosa oil were geraniol (51.31 %) and neryl acetate (24.92 %). Also, D-limonene (3.0 %), limonene dioxide (3.52 %) and linalool (2.14 %) were also reported. Lemongrass oil showed the presence of citral (41.64 %), neral (21.67 %) and neryl methyl ether (14.55 %) as the active ingredients. A wide range of compounds in patchouli oil, such as sychelles (21.03 %), α -guanine (19.0 %), α -guanine (15.42 %) and patchouli alcohol (12.97 %) were recorded as the main compounds. In turmeric leaf oil, the major components were terpinolene (39.41 %), p-cymenol (14.28 %) and p-cymene (9.02 %) (Table 4).

Table 4. Major components of the selected essential oils by GC-MS analysis.

| Essential Oils | Major Components | Retention Time | Conc. (%) |
|----------------|------------------------|----------------|-----------|
| Lemongrass | Citral | 21.81 | 41.64 |
| | Neral | 21.14 | 21.67 |
| | Neryl Methyl Ether | 23.20 | 14.55 |
| | Sulcatone | 14.06 | 6.77 |
| | Geraniol | 21.38 | 1.71 |
| | Citronellal | 18.98 | 1.50 |
| Palmarosa | Geranial | 21.50 | 51.31 |
| | Neryl Acetate | 24.14 | 24.92 |
| | Limonene Dioxide | 25.52 | 3.53 |
| | D-Limonene | 15.46 | 3.00 |
| | Linalool | 17.61 | 2.14 |
| | Nerol | 21.80 | 1.70 |
| Patchouli | Citral | 21.12 | 0.62 |
| | Seychellene | 25.83 | 21.03 |
| | α -guajene | 26.77 | 19.00 |
| | α -guaiene | 25.45 | 15.41 |
| | Patchouli alcohol | 30.01 | 12.97 |
| Turmeric | α -cedrene | 26.06 | 9.94 |
| | Terpinolen | 17.29 | 39.41 |
| | p-cymenol | 19.96 | 14.27 |
| | p-cymene | 15.33 | 9.021 |
| | Eucalyptol | 15.61 | 8.03 |
| | α -phellandrene | 14.71 | 5.91 |

Essential oils are rich sources of volatile chemicals, primarily monoterpenes and sesquiterpenes (37, 38). Lemongrass oil contains citral, nerol, geraniol and linalool (39), which are analogous to the components obtained in the present study. The components of the palmarosa oil examined in this research are geranyl acetate, geraniol and caryophyllene oxide (40), similar to the palmarosa oil from Benin.

In the case of turmeric, α - pinene, β - pinene, β -myrcene, p-cymene and D-limonene were found similar to the previous report on turmeric leaf essential oil (41). It was noticed that certain elements in patchouli oil, such as patchouli alcohol, α -guanine and seychelles were similar to the components reported in patchouli oil from Indonesia (42).

Antifungal activities of essential oil

Essential oils are known for their broad-spectrum antifungal (43) and eco-friendly properties (44). The mode of action of essential oil components against pathogens includes inhibiting biochemical pathways and protective enzymes, altering gene expression, cellular respiration and energy metabolism (45). In this study, the antifungal activities of the investigated essential oils have been observed against the identified fungal pathogens.

Whereas F1B and F3BR were only slightly affected, F4BW and F5G's growth was entirely inhibited by palma-

rosa essential oil at 3 to 15 μ l/ml (Table 5). The MIC for F4BW and F5G was found to be 3 μ l/ml. On the other hand, MICs for F1B and F3BR were not found in the tested ranges. For F4BW, MFC was detected at 3 μ l/ml; however, MFC was not detected for the other isolates.

Table 5. Mycelial growth inhibition percentage due to palmarosa essential oil.

| Isolate | Conc(μ l/ml) | | | | |
|---------|-------------------|----------------|----------------|----------------|----------------|
| | 3 | 6 | 9 | 12 | 15 |
| F1B | 75.4 \pm 0.4 | 81.1 \pm 1.7 | 86.4 \pm 0 | 86.4 \pm 0 | 86.4 \pm 0 |
| F3BR | 76.5 \pm 0.5 | 79.6 \pm 1.0 | 86.2 \pm 1.5 | 86.2 \pm 1.5 | 86.2 \pm 1.5 |
| F4BW | 100 | 100 | 100 | 100 | 100 |
| F5G | 100 | 100 | 100 | 100 | 100 |

The values represent the mean \pm standard error of the percentage of triplicate. The significance of the findings was determined by analysis of variance (one-way ANOVA). P-value was <0.05.

The antifungal activity of palmarosa essential oil has been reported in numerous studies. It was reported that palmarosa oil damages the plasma membrane of *Penicillium expansum* (46). Similarly, a study analyzed the essential oil and reported it as a potent mold inhibitor consisting of geraniol (43.80 %), α -ocimene (10.75 %), geranyl acetate (6.35 %), 2,6-octadienal, 3,7-dimethyl-(E) (5.83 %) as major volatile constituents (47).

Lemongrass essential oil also inhibited the fungal (F4BW and F5G) mycelia growth completely at ranges from 3 to 15 μ l/ml, whereas F1B was inhibited completely at 12 μ l/ml. Complete inhibition of F3BR was found at 9 μ l/ml among the tested concentrations (Table 6). MIC for F4BW, F1B and F3BR was 3, 12 and 9 μ l/ml respectively.

Table 6. Mycelial growth inhibition percentage due to lemongrass essential oil.

| Isolate | Conc(μ l/ml) | | | | |
|---------|-------------------|----------------|--------------|-----|-----|
| | 3 | 6 | 9 | 12 | 15 |
| F1B | 83.4 \pm 1.6 | 91.7 \pm 0.4 | 93 \pm 0.4 | 100 | 100 |
| F3BR | 31.4 \pm 1.3 | 72.2 \pm 1.0 | 100 | 100 | 100 |
| F4BW | 100 | 100 | 100 | 100 | 100 |
| F5G | 100 | 100 | 100 | 100 | 100 |

The values represent the mean \pm standard error of the percentage of triplicate. The significance of the findings was determined by analysis of variance (one-way ANOVA). P-value was <0.05.

A study revealed that lemongrass essential oil has antifungal activity against various postharvest pathogens such as *Aspergillus niger*, *Rhizopus stolonifer*, *Cladosporium herbarium*, *Botrytis cinerea* and *Colletotrichum coccodes* (22). The antifungal action is attributed to 2 isomeric acyclic monoterpene aldehydes (geranial and neral) of citral (48, 49). It was reported that geraniol partially disrupted the integrity of cell membranes, leading the higher electrical conductivity to accelerate cell death (50). It was demonstrated that the antifungal property of citral was achieved by modulating growth-related genes in *A. flavus* (50). In this study, it was simulated that citral and geraniol might have inhibited fungal growth by interfering with

related genes, changing cell membrane permeability or inducing intracellular accumulation of reactive oxygen species (ROS). A study reported similar action by citral and geraniol (51). Membrane proteins interact with monoterpenes, which cause structural changes in hyphae and plasma membranes, leading to fungal cell death (52).

Turmeric leaf oil also partially inhibited all the isolates except F4BW, which was inhibited slightly at 15 µl/ml (Table 7). It was stated that turmeric leaf oil had α-phellandrene (46.7 %), α-terpinolene (17.3 %) components, which were found to be a potent antifungal agent (53). In this research, the turmeric leaf oil yielded α-phellandrene (5.9 %) and terpinolene (39.4 %); nevertheless, its efficacy against isolates of spoilage fungi was limited.

Table 7. Mycelial growth inhibition percentage due to turmeric leaf essential oil.

| Isolate | Conc(µl/ml) | | | | |
|---------|-------------|----------|----------|----------|----------|
| | 3 | 6 | 9 | 12 | 15 |
| F1B | 15.3±0.5 | 24.7±0.3 | 43.1±1.6 | 51.1±0.7 | 57.3±0.7 |
| F3BR | 18.6±1.3 | 33±1.4 | 45.1±0.9 | 51.6±0.5 | 53.5±0.4 |
| F4BW | 0 | 0 | 0 | 0 | 19.1±1.4 |
| F5G | 11.7±0.7 | 32.9±0.3 | 41.3±0.3 | 62.1±0.1 | 65±0.3 |

The values represent the mean ± standard error of the percentage of triplicate. The significance of the findings was determined by analysis of variance (one-way ANOVA). P-value was <0.05.

ited.

Patchouli essential oil inhibited all the isolates to a lesser extent, but no MIC or MFC was found among the tested concentrations (Table 8). Patchouli essential oil is known to exert good antibacterial activity (54). However, patchouli oil extracted in this study had no significant efficacy against the isolated spoilage fungi.

Table 8. Mycelial growth inhibition percentage due to patchouli essential oil.

| Isolate | Conc(µl/ml) | | | | |
|---------|-------------|-----------|----------|----------|-----------|
| | 3 | 6 | 9 | 12 | 15 |
| F1B | 59.8±0.9 | 71.9±0.4 | 78.6±0.3 | 80.1±0.3 | 86.01±0.1 |
| F3BR | 52.5±0.5 | 56.8±0.5 | 63±0.3 | 67±0.5 | 71.5±0.6 |
| F4BW | 21.6±0.6 | 65.43±0.5 | 73±0.6 | 80.4±0.4 | 82.6±0.3 |
| F5G | 43.7±0.3 | 47.3±0.3 | 55.5±0.1 | 58.4±0.2 | 64.07±0.2 |

The values represent the mean ± standard error of the percentage of triplicate. The significance of the findings was determined by analysis of variance (one-way ANOVA). P value was <0.05.

cacy against the isolated spoilage fungi.

Conclusion

According to the present research, *A. welwitschiae* (F1B) was found to be the most pathogenic fungus (80 %) for tomato fruits among the isolated fungi after 21 days. Additionally, *Rhizopus arrhizus* (F4BW) was observed to cause fast decomposition (26 %) of tomatoes within 5 days. The investigation demonstrates that essential oils have promising ingredients to control spoilage fungi in tomatoes.

Lemongrass oil completely inhibited the mycelial growth of *A. welwitschiae* (F1B) and *Rhizopus arrhizus* (F4BW). Furthermore, palmarosa oil showed fungicidal activity against *Rhizopus arrhizus* (F4BW). Therefore, palmarosa and lemongrass oils were found to be more effective than patchouli and turmeric leaf oils against pathogenic fungi. However, the primary barriers to the use of EOs for food preservation include their safety limitations, noticeable organoleptic effects and contamination by chemicals found in goods like pesticides. To conclude, further investigations are needed to determine suitable ways of application, toxicity, responsible antifungal compounds and dosage limits of such essential oils as eco-friendly food preservatives.

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Authors' contributions

RS and FSC performed experimental work and prepared the manuscript, MSH conducted the statistical analysis, FY and JF contributed to experimental work and manuscript editing, G.M. MR carried out the GC-MS analysis, MSR and NCN completed the essential oil analysis. HBR and MM revised the manuscript. SI designed the project, prepared the manuscript and supervised the overall work. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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Supplementary data

Supplementary Tables

Table 1. Palmarosa essential oil composition

Table 2. Lemongrass essential oil composition

Table 3. Patchouli essential oil composition

Table 4. Turmeric leaf essential oil composition

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