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Effect of essential oils on *Aspergillus* spore germination, growth and mycotoxin production: a potential source of botanical food preservative

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PEER REVIEW

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Comments

This study is an excellent piece of work where the authors investigated the preservative effect of essential oils against food contaminating toxigenic *Aspergillus* fungi. The *in vitro* antifungal and antiaflatoxigenic activity of essential oils was evaluated using poisoned food techniques, spore germination assay, agar dilution assay, and aflatoxin arresting assay on toxigenic strains of *Aspergillus* species.
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ABSTRACT

Objective: To investigate effect of essential oils on *Aspergillus* spore germination, growth and mycotoxin production.

Method: *In vitro* antifungal and antiaflatoxigenic activity of essential oils was carried out using poisoned food techniques, spore germination assay, agar dilution assay, and aflatoxin arresting assay on toxigenic strains of *Aspergillus* species.

Results: *Cymbopogon martinii, Foeniculum vulgare* and *Trachyspermum ammi* (*T. ammi*) essential oils were tested against toxicogenic isolates of *Aspergillus* species. *T. ammi* oil showed highest antifungal activity. Absolute mycelial inhibition was recorded at 1 μl/mL by essential oils of *T. ammi*. The oil also showed, complete inhibition of spore germination at a concentration of 2 μl/mL. In addition, *T. ammi* oil showed significant antiaflatoxigenic potency by totally inhibiting aflatoxin production from *Aspergillus niger* and *Aspergillus flavus* at 0.5 and 0.75 μl/mL, respectively. *Cymbopogon martinii, Foeniculum vulgare* and *T. ammi* oils as antifungal were found superior over synthetic preservative. Moreover, a concentration of 5 336.297 μl/kg body weight was recorded for LC50 on mice indicating the low mammalian toxicity and strengthening its traditional reputations.

Conclusions: In conclusion, the essential oils from *T. ammi* can be a potential source of safe natural food preservative for food commodities contamination by storage fungi.

KEYWORDS

Aspergillus species, Essential oils, Food spoilage, Mycotoxin, Preservatives

1. Introduction

Despite the advancement food science and the technology of food production, diseases caused by foodborne fungal pathogens are still the major public health problems and quarter of worlds' food commodities have been wasted due to the contamination by toxic fungi or by fungal metabolic products[1,2]. Improper storage conditions offer favorable environment for the growth of *Aspergillus* spp and production of mycotoxins[2]. Consumptions of such contaminated food lead to serious cases of illness and mycotoxicoses[3,4]. Among these, aflatoxicosis causes acute hemorrhage, acute liver damage, edema, and death and chronic toxicological effect of cancer, mutagenicity, immune suppression, birth defects, estrogenic, gastrointenial, urogenital, vascular, kidney and nervous system disorder[1,2,5]. In Africa particularly, in parts of sub-Saharan Africa about 250 000 hepatocarcinoma related deaths occur annually due to aflatoxin ingestion alone[1,4].

Management of food stuffs contaminations are required to ensure that food commodities remain safe and uncontaminated throughout the supply chain (from 'farm to plate')[2]. Several synthetic preservatives have been

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effectively used in management of food contamination by *Aspergillus* spp. But their continuous application has led to the development of fungal resistance[6], number of environmental and health problems[7-9]; hormonal imbalance and spermatotoxicity and also some individuals produce allergic reactions to these substances[10,11]. However, natural products could potentially serve as effective alternatives of synthetic chemicals for the control of food contamination by *Aspergillus* spp[12,13]. Among natural products, essential oils (EOs) of aromatic plant are gaining interest as food additives and widely accepted by consumers because of their relatively low toxicity, high volatility, transient nature and biodegradability[14,15]. European Union allowed the use of EOs in food and aromatherapy[16]. So, EOs with antimicrobial activity are possible candidates for the preservativation of food commodities against *Aspergillus* spp[17].

Cymbopogon martinii (*C. martinii*) L., *Foeniculum vulgare* (*F. vulgare*) Miller and *Trachyspermum ammi* (*T. ammi*) L. Sprague ex Turrill are medicinal aromatic plant of Ethiopia. They are used traditionally as food additives and also for the treatment of various diseases[18]. However, there is no reliable evidence that indicated these plants EOs have fungitoxic and antiaflatoxigenic potential against aflatoxigenic *Aspergillus* spp in Ethiopia. The aim of this study was to evaluate the effect of EOs on growth, spore and mycotoxin production of *Aspergillus* spp that could alternate synthetic chemical preservatives.

2. Materials and methods

2.1. Chemicals and media

Our media, chemical and solvents used in the study were obtained from different companies in different countries: Peptone Dextrose Agar was obtained from Himedia Laboratories Pvt. Ltd., India; Sabouraud Dextrose Agar from Oxoid Ltd., England; Sabouraud Dextrose Broth from Defco, Ltd., England; sucrose and yeast extract from Labort fine Chem Pvt. Ltd, India; Aflatoxin Mix Kit-M from Supelco, USA; anhydrous sodium sulphate, MgSO4.7H2O, potassium hydroxide, vanillin and silica gel 60 thin layer chromatography (TLC) plate 0.2 mm from Merck-Schuchardt, Germany; potasium nitrate from Rhone Poulenc, US; sodium benzoate from Codex® Farmacopia, Italy; thymol, chloroform, ethyl acetate, sulphuric acid, tween 20 and tween 80 from Sigma-Aldrich Chemie, Germany; acetone from Labort Fine Chem Pvt. Ltd, India; ethanol absolute and methanol from Finkem, India; and Tolune from AnalaR®, England.

2.2. Plant material collection identification and extraction

Different parts of the test plants of *C. martinii* (aerial part) was collected from the botanical garden of TMMRD, the Ethiopian Health and Nutrition Research Institute (EHNRI), Ethiopia; *F. vulgare* (leaf lamina and leaf sheath) were collected from Shashamane, Ethiopia; and *T. ammi* (fruits) was collected from Tepi, Ethiopia. Following collection, the identities of plant materials were confirmed by taxonomist and botanist in Traditional and Modern Drug Research Department of EHNRI. Fresh areal part of *C. martinii*, *F. vulgare* and dried fruits of *T. ammi* (250 g) were placed in a 5 L round-bottom distillation flask and the plant material was wetted with 3 L distilled water. The EOs were obtained by hydro-distillation using Clevenger-type apparatus for continuous 3 h. The volatile oil was taken from the upper layer. The excess aqueous layer was further portioned using dichloromethane to extract and enrich the EO from the water layer. The organic layer (dichloromethane extract) was filtered and dried with anhydrous sodium sulfate and concentrated using rotary evaporator to give the crude EO.

2.3. Phytochemical screening of the EOs

2.3.1. TLC analysis

Following the extraction of EOs that are intended for biological assay, a portion of EOs are subjected to TLC (pre coated silica gel G60 F254) finger print analyses for preliminary qualitative phytochemical screenings of the most active EOs for various secondary metabolites that have antimicrobial activity. Wagner & Bladts' procedures for plant drug analysis were used for the development of chromatogram and for the identification of major secondary metabolites responsible for biological activity[19].

2.3.2. Gas chromatographic (GC)-analysis

GC analysis of the oil of *T. ammi* was performed on a Shimadzu GC-2010 system, with split mode. The column used was a ZB-1MS equivalent to OV-1, fused silica capillary column 30 m×0.25 mm i.d., film thickness 0.25 μm, coated with 5% diphenyl-95% polydimethylsiloxane, operated with the following oven temperature programme: 50 °C, held for 2 min, rising at 3 °C to 210 °C/min. Injection temperature and volume, 250 °C and 1.0 μL, respectively; injection mode, split; split ratio, 10:1; carrier gas, nitrogen at 65.2 cm/s linear velocity and inlet pressure 100 KPa; detector temperature, 270 °C; nitrogen flow rate, 52.1 mL/min; air flow rate, 400 mL/min; make-up 32, (H2/air) flow rate 40 mL/min; sampling rate, 40 MS/s.

2.4. Test organisms

Aflatoxicogenic strains of *Aspergillus flavus* (*A. flavus*) and *Aspergillus niger* (*A. niger*) were selected for this study. The strains were isolated from food commodities that were collected from different local markets in Addis Ababa prior to the study. In addition, standard strains of *A. flavus* (ATCC 13697) and *A. niger* (ATCC 10535) were used in the study as a control. The standard strains were obtained from Microbiology Laboratory, Traditional and Modern Medicine Research Directorate, EHNRI.

2.5. Antifungal activity

2.5.1. Determination of sporicidal activity

Sporicidal activity of *C. martinii*, *F. vulgare* and *T. ammi* were conducted using spore germination assay according to standard reference methods[20]. The test organisms were grown on PDA medium for sporulation and spores were harvested when the cultures were fully sporulated, which was achieved after 10 d of incubation. Spores were collected by adding 5 mL of sterile water containing 0.1% (v/v) tween 80 (for better spore separation) to each Petri dish and rubbing the surface with a sterile L-shaped spreader (3 times). The suspension was collected and then centrifuged at room temperature at 2000 r/min for