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Discrete and combined effects of Ylang-Ylang (*Cananga odorata*) essential oil and gamma irradiation on growth and mycotoxins production by *Fusarium graminearum* in maize

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ABSTRACT

In the present study, discrete and combined inhibitory effects of *Cananga odorata* essential oil (COEO) and irradiation were established on growth and production of deoxynivalenol (DON) and zearalenone (ZEA) by *Fusarium graminearum* in maize kernels. Chemical profile of COEO was characterized by GC-MS and a total of 35 chemical constituents were identified, and major compounds were linalool (29.15%), germacrene-D (11.82%), and thymol (8.45%). The COEO and irradiation have inhibited the fungal growth and mycotoxins at 3.9 mg/g and 7.5 kGy, respectively. Distinct inhibitory activity of COEO and irradiation on fungal growth and mycotoxins was assessed by constructing linear regression curves. Regression models of COEO and irradiation have presented good coefficients of determination (R^2) of 0.9886 and 0.9798 for fungal growth (log CFU), 0.97 and 0.9622 for DON, and 0.9811 and 0.9807 for ZEA, respectively. The linear regression models concluded that COEO and irradiation have dose-dependent inhibitory effect on fungal growth and mycotoxins. Further, combined inhibitory effect of COEO and irradiation on fungal growth and mycotoxins was assessed by checkerboard method. The combined treatment of COEO and irradiation was too shown decent coefficients of determination (R^2) and found greatly effective. The combined treatments of COEO and irradiation have inhibited the fungal growth and mycotoxins in stored maize kernels much below than their discrete inhibitory levels, and it was noticed at 2.5 mg/g of COEO and 4 kGy of irradiation. The study concluded that combination of essential oil and irradiation could be highly efficient decontamination technique to diminish the fungal growth and mycotoxins in agricultural commodities.

1. Introduction

Contemporary research has intensified much awareness about the prevalence of foodborne pathogens and its toxins in agricultural commodities. Particularly, fungal infestations and mycotoxins contamination of agricultural commodities is one of the utmost concerns for food safety and quality (Pitt & Hocking, 2009). The Food and Agriculture Organization (FAO) of United Nations estimate that about one-quarter of agricultural commodities were pretentious with fungi and mycotoxins worldwide (Jackson & Jablonski, 2004). The fungal infestation and mycotoxin contamination takes place due to unhygienic and inappropriate practices at various stages of pre- and post-harvesting sessions, i.e. growing, harvesting, storage, transportation, processing, and distribution (da Cruz Cabral, Pinto, & Patriarca, 2013).

The foremost mycotoxigenic fungal species connected with agricultural products are *Aspergillus*, *Penicillium*, *Fusarium,* and *Alternaria*. Among these mycotoxigenic fungi, *F. graminearum* has turned out one of the utmost worries of food safety and quality. The *F. graminearum* is accountable for stalk rot and Gibberella ear rot in maize, and Fusarium head blight (FHB) in barley, oats, and wheat, and annually liable for loss of 50% yield and billion dollars worldwide (Pasquali et al., 2016). The *F. graminearum* could produce prominent mycotoxins, i.e. deoxynivalenol (DON) and zearalenone (ZEA) under diverse climatic conditions (Mudili et al., 2014). The DON and ZEA are known to cause

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various deleterious effects, i.e. mutagenicity, dermal toxicity, neurotoxicity, gastrointestinal toxicity, nephrotoxicity, reproductive and developmental toxicity, and hepatotoxicity in humans and livestock called mycotoxicosis (Richard, 2007; Sobrova et al., 2010; Zinedine, Soriano, Molto, & Manes, 2007). Moreover, International Agency for Research on Cancer (IARC) has evaluated the carcinogenic hazard posed by DON and ZEA under in vivo conditions and categorized into Group 3 carcinogens (IARC, 1993). In view of these toxicological complications, regulatory agencies and many nations have recommended stringent legislative levels for DON and ZEA mycotoxins in food and feed matrices. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established a Provisional Maximum Tolerable Daily Intake (PMTDI) for DON and ZEA as 1 and 0.50 μg/kg body weight per day, respectively (JECFA, 2011). The European Union (EU) has set a maximum permitted limit for DON and ZEA in unprocessed maize at 0.35 and 1.75 mg/kg, respectively. Further, EU has set a maximum permitted limit for DON in unprocessed durum wheat and oats, and other unprocessed cereals (excluding durum wheat, oats, and maize) at 1.75 and 1.25 mg/kg, respectively. The maximum permitted levels for ZEA in unprocessed cereals excluding maize is 0.1 mg/kg (European Commission, 2006).

In last few decades, quite a lot of incidences of *F. graminearum*, DON, and ZEA contamination were recorded worldwide in a wide range of agricultural products, such as maize, wheat, rice, sorghum, vegetables and its oils, spices, etc., For instance, Mallmann et al. (2017) has surveyed the incidence of FHB, DON, and ZEA in Brazil during 2008–2015 and noticed that 67% and 41% of barley, 73% and 38% of wheat samples were positive for DON and ZEA contamination, respectively. Similarly, Pleadin et al. (2017) in Croatia has noticed massive contamination of DON and ZEA in unprocessed cereals and soybean in the range of 1461 ± 2265 and 656 ± 853 for 2014, and 2687 ± 2731 and 1140 ± 1630 μg/kg for 2015, respectively. In Serbia, Kos et al. (2017) has assessed the influence of weather on the prevalence of DON in maize during 2013, 2014 and 2015 and noticed in the range of 260.1–1388, 260.4–9050 and 252.3–6280 μg/kg, respectively. In middle and eastern parts of Europe, Tima, Brückner, Mohácsi-Farkas & Kiskó (2016) has surveyed the cereal kernels and noticed 86% of DON and 41% of ZEA contamination. From the different province of China, Wu et al. (2016) has determined 93.9% of DON and 92.3% of ZEA contamination in a wide range of feed ingredients. In one of our previous study also reported the incidence of *F. graminearum* and 72–94 μg/kg of DON attendance in freshly harvested maize kernels originated from the Southern region of India (Mudili et al., 2014). These situation's apprehensions the prevalence of *F. graminearum*, DON and ZEA contamination worldwide and demands efficient strategy to overcome these issues.

In the present scenario, the main challenge is to deliver fungi and mycotoxin-free food to the consumer. Mycotoxins are heat stable and resistant to various food-processing techniques and very hard to destroy in food (Pitt & Hocking, 2009). Therefore, the best alternative is to reduce/eliminate the possibilities of mycotoxins inflowing the food chains (Magan & Olsen, 2004). The food industry has offered numerous decontamination techniques to safeguard food from fungi and mycotoxins and many of them were inadequate, undesirable and not acceptable. Therefore, there is still a huge demand for newer or improvement of existing technologies into efficient and innovative (Kalagatur et al., 2018; Sellamani et al., 2016). The application of synthetic antifungal agents in food is not at all tolerable because of its harmful health effects, environmental pollution and development of drug-resistant fungi (da Cruz Cabral et al., 2013). The chemical decontamination techniques bring undesirable changes in food, i.e. texture, aroma, and taste, and could form toxic by-products (Pitt & Hocking, 2009). The physical decontamination techniques, particularly gamma radiation treatment is substantially potent and has quick decontamination effect (Calado, Venâncio, & Abrunhosa, 2014). The gamma rays are extremely penetrative electromagnetic radiations and have high energy of 100 KeV or above. It takes only about 10^{-6} s for radiolysis of water molecules and produces highly reactive molecules by several recombination and cross-combination reactions (Le Caër, 2011). These molecules detrimentally interact with biomolecules (DNA, proteins, and lipids), disrupt cell membrane fluidity and cell wall structure, and initiate the apoptotic death of fungi (Calado et al., 2014). Furthermore, plant-based antimicrobials, i.e. crude extracts, essential oils, and phytocompounds are highly satisfactory due to its environment-friendly and beneficial health effects (Kalagatur, Ghosh, Sundararaj, & Mudili, 2018; Kalagatur et al., 2017; Muniyandi et al., 2017; George et al., 2016; da Cruz Cabral et al., 2013). In this context, an innovative combination of essential oil and gamma radiation technique could be highly effective for controlling the fungi and mycotoxins in food.

Accordingly, *Cananga odorata* essential oil (COEO) was selected with the combination of gamma irradiation to assess the inhibitory effect on fungal growth and mycotoxins. *C. odorata* is commonly known as Ylang-Ylang and belong to family Annonaceae. It is a native of tropical Asia and nowadays extensively distributed in Southern Asian countries (Tan et al., 2015). Since ancient times, it is recognized as herbal plant and its essential oil is popularly used to treat a wide number of ailments, such as cold, asthma, inflammation, fever, wounds, microbial infections, etc., (Srivastava & Bajaj, 2014). It is also one of the most commonly used spices in a variety of cuisines and beverages as food seasonings worldwide.

In the present study, COEO was extracted from flower buds by hydro-distillation technique and chemical profile was characterized by GC-MS analysis. The discrete and combined inhibitory effect of COEO and irradiation on fungal growth, and production of DON and ZEA of *F. graminearum* were assessed in stored maize kernels by the checkerboard method. The obtained data was analyzed to fit linear regression curve, and its goodness of fit was judged by coefficients of determination.

2. Materials and methods

2.1. Chemicals and reagents

Sabouraud dextrose agar (SDA) and peptone were obtained from HiMedia (Mumbai, India). Acetonitrile, HPLC grade water, and other chemicals were obtained from Merck Millipore (Bengaluru, India). Certified standards of DON and ZEA were obtained from Sigma-Aldrich (Bengaluru, India). The immunoaffinity clean-up columns of DONtest WB for DON and ZearalaTest WB for ZEA were purchased from Vicam (Waters, USA).

2.2. Fungal culture conditions

The mycotoxigenic *F. graminearum* (DFRL FgM: 18) positive for the synthesis of DON and ZEA, which was isolated from maize kernels in our previous study of Mudili et al. (2014) was grown on SDA Petri plate for 7 days at 28 °C. The fungal spores were picked from the SDA Petri plates in sterilized peptone water containing 0.01% Tween 80 (v/v) with a gentle scrape and filtered through sterile gauze to get rid of debris. The spore count was appraised by hemocytometer and sum was adjusted about to 10^6 spores/mL and spore suspension was used in further experiments.

2.3. Extraction and chemical profile of essential oil

The flower buds of *Cananga odorata* were collected from Ooty, Tamil Nadu, India. The plant material was safeguarded (voucher no: PEO 52) in Food Microbiology Division, Defence Food Research Laboratory, Mysuru, India. The flower buds were washed with distilled water and air dried for two weeks under shade at room temperature (25 ± 2 °C) and crushed into fine powder. The essential oil was extracted from 250 g powder by distillation technique employing a Clevenger type