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Radiosensitization of *Aspergillus niger* and *Penicillium chrysogenum* using basil essential oil and ionizing radiation for food decontamination

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ABSTRACT

Stored products may be contaminated by pathogenic fungi such as Fusarium, Aspergillus and Penicillum. Fumigation with plant essential oil (EO) and irradiation treatment are options to control spoilage organisms. Basil essential oil and irradiation were tested alone and in combination for their antifungal effects in rice. Minimum Inhibitory Concentration (MIC) of basil EO was found to be 0.1% (v/v) against *Aspergillus niger* and *Penicillium chrysogenum* after 48 h. Radiosensitization of *A. niger* and *P. chrysogenum* in presence of 1% or 2% (v/v) basil EO was evaluated *in vitro* and *in situ*. At 1 and 2% of basil EO, the *in vitro* D_{10} value was 0.43 and 0.31 kGy respectively for *A. niger* and 0.44 and 0.34 kGy respectively for *P. chrysogenum*. In inoculated rice, D_{10} values for controls (sample without EO) were 0.67 and 0.63 kGy for *A. niger* and *P. chrysogenum* respectively, and the values were decreased at higher EO concentrations. For *A. niger*, a 2% (v/wt) basil EO alone caused a 0.42 to 1.18 log reduction on days 1 and 14 respectively, whereas treatment with 2 kGy radiation alone caused a 2.18 log reduction. The combined treatments resulted in a 4.6 log reduction of *A. niger* after 14 days of storage. For *P. chrysogenum*, 2% basil EO alone caused a 0.76 and a 1.12 log reduction on days 1 and 14 respectively, whereas a 2 kGy radiation dose caused a 2.41 log reduction. The combined treatments resulted in a 5.0 log reduction of *P. chrysogenum* after 14 days of storage. The findings demonstrated the potential of basil EO as antifungal agent and its efficacy to increase the radiosensitivity of *A. niger* and *P. chrysogenum* during irradiation treatment.

1. Introduction

Pathogenic molds such as *Fusarium* spp., *Aspergillus* spp., and *Penicillium* spp. have been reported as causal agents of food spoilage and food-borne diseases (Betts, Linton, & Betteridge, 1999; Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2007). These fungi can contaminate foods from cultivation to harvest, and during transportation and storage. Fumigation with plant essential oils (EO) and irradiation treatment are fungicidal options to control spoilage caused by disease microorganisms in food. In the case of grains, transportation may include long overseas journeys, during which the moisture content of the dried grains can increase to a suitable level for the growth of xerophillic fungi. The metabolic moisture and heat resulting from respiration of fungi growing on grain creates an ideal environment for the growth of less xerotolerant fungi as well. This chain reaction can result in massive colonization by various types of fungi in bulk grain (Makunh, Gbodit, Akanyao, Salakoe, & Ogbadug, 2007).

The effects of fungal invasion of grain include development of visible mold, discoloration, unpleasant odor, chemical and nutritional changes, loss of quality and production of mycotoxins. Applying synthetic fungicides in the field can control many pathogenic and toxicogenic fungi (Chen, Moore, & Nesnow, 2008). However, due to adverse environmental effects and resistance development, alternative control methods are needed (Deising, Reimann, & Pascholati, 2008; Kabera, Gasogo, Uwamariya, Ugirinshuti, & Nyetera, 2011). Plant EOs contain a wide array of aromatic compounds that give plants a distinctive odor, flavor or scent. These aromas are complex mixtures of a large number of constituents, but mainly consist of monoterpenes and sesquiterpenes and their oxygenated derivatives (alcohols, aldehydes, esters, ethers, ketones, phenols and oxides) in variable ratios (Boumail

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et al., 2013; Ebadollahi & Mahboubi, 2011; Lee, Choi, Lee, & Park, 2001). Basil (*Ocimum basilicum* L.) EO belonging to the Lamiaceae family is known to exhibit antimicrobial and antifungal properties, but studies on combined antifungal effects of basil EO and irradiation are limited (Lashowicz et al., 1998).

Irradiation is another alternative to preserve food items and ensure they are free from pathogenic microorganisms. Irradiation has been considered as a safe and effective technology by the World Health Organization (WHO), Food and Agricultural Organization (FAO) and the International Atomic Energy Agency (IAEA) (IAEA., 2004). Several studies have reported that molds are sensitive to ionizing radiation and their mycotoxin production decreases after irradiation treatment (Rustom, 1997; Youssef, Mahrous, & Aziz, 1999). The objectives of this study are to 1) determine the antifungal properties of basil EO against two molds, *Aspergillus niger* and *Penicillium chrysogenum* and 2) evaluate their radiosensitivity during combined treatment with basil EO and ionizing radiation.

2. Materials and methods

2.1. Basil EO

Ocimum basilicum, (100% purity) was obtained from Robert & Fils, Ghislenghien, *Belgium*.

Chemical analysis provided by the manufacturer has indicated that the tested basil oil contained 77.6% estragole (methyl chavicol) and 20.30% linalool as major components.

2.2. Fungal inocula and assay media

A. niger ATCC 1015 and *P. chrysogenum* ATCC 10106 were grown and maintained in potato dextrose broth (PDB, Difco, Becton Dickinson) containing glycerol (10% v/v). Prior to each experiment stock cultures were propagated through two consecutive 48 h growth cycles in potato dextrose broth at 28 °C ± 2°. The fungi were then pre-cultured in PDA for 3 days at 28 °C ± 2°. Conidia were isolated from the agar media using sterile saline containing 0.05% Tween 80. Mycelia were removed by filtration through gauze, and the filtrate concentration was adjusted to 1×10^6 conidia/ml for *in vitro* experiments on potato dextrose broth (PDB) media and *in situ* experiments on packaged rice.

2.3. Preliminary study

The antifungal activity of basil EO was evaluated by the agar disc diffusion and volatilization methods (Benkeblia, 2004; Kordaly et al., 2005; Lopez, Sanchez, Batlle, & Nerin, 2005). Both methods showed notable antifungal efficiency of basil EO against *A. niger* and *P. chrysogenum*. The agar diffusion test using different concentration of basil EO showed that a volume of 10 μl of 5% diluted basil EO produced clear inhibition zones of (10 ± 0.6) mm diameter against *A. niger* and (13 ± 0.4) mm diameter against *P. chrysogenum*. In the volatilization assay a quantity of 30 μl of 5% basil EO was required to produce the same inhibition effect as that of the agar diffusion method against *A. niger and P. chrysogenum*. The diffusion assay was more sensitive than the vapor assay with the same concentration of basil EO (data not shown).

2.4. Determination of MIC

The agar dilution method described by Bansod and Rai (2008) was adopted with some modifications. Dilutions of basil EO ranging from 0.5% (v/v) to 0.005% (v/v) were prepared in PDA in the presence of Tween 20 (0.05% v/v) to enhance oil solubility. A sample of 20 ml of the diluted EO was plated onto Petri dishes and allowed to solidify at room temperature. PDA media containing Tween 20 (without EO) was used as a control. The prepared PDA plates were inoculated with 100 μl of *A. niger* and *P. chrysogenum* representing 1×10^6 conidia/ml. The fungal inocula were spread homogenously using glass beads and the plates were tightly sealed with sterile parafilm to prevent evaporation of EO. The plates were incubated at 28 °C ± 2 °C for 48–96 h and colonies were checked twice a day. Minimum inhibitory concentrations (MICs) were determined as the lowest concentration of EO that inhibited the growth of each microorganism on the agar plate.

2.5. In vitro radiosensitization of A. niger and P. chrysogenum

The viability of irradiated and non-irradiated spores were determined by a dilution plate method in three replicates (up to $^{10-6}$ dilution) after 72 h incubation time on PDB media. Concentrations of 0, 1% and 2% of basil EO were added before determining the radiosensitivity of *A. niger* and *P. chrysogenum*. The microbial colonies were counted following the plate count method described by (Dubey & Maheshwari, 2005). After 24 h, all samples were irradiated at the Canadian Irradiation Center in an UC-15A underwater calibrator (NORDION, Kanata, Canada) equipped with a ^{60}Co source and having a dose rate of 19 kGy/h. Samples were irradiated at room temperature at eight doses ranging from 0 to 4 kGy. Samples without basil EO were included as controls.

2.6. Inoculation and basil oil treatment of packaged rice (in situ test)

A quantity of 30 g of white long grain rice (Nu pak, Shah Trading Company Limited, Scarborough, Ontario) was inoculated with 200 μl of (1×10^6 conidia/ml) *A. niger* or *P. chrysogenum* in sterile plastic bags. A quantity of 1%, 2% or 4% (v/wt) basil EO was added to a sterile sponge cube of dimension 5 × 5 × 5 cm placed inside a cup. The sponge cubes were covered with muslin screen to ensure that the rice grains were not in direct contact with the essential oil, in view of determining the vapor effect of basil EO. The oil infused sponges in muslin were placed in the sterile plastic bags containing rice inoculated with the fungal conidia to evaluate the vapor effect of basil EO during storage. The samples were grouped into two subsets with one to receive irradiation and one not to receive irradiation. The samples were incubated at 30 °C for 14 days. The humidity inside the incubator was monitored and maintained constant at 65% throughout the experiment. The microbiological analyses of the stored rice grain were carried out after 1, 7 and 14 days of storage.

2.7. Microbiological analysis

Microbiological analyses of all the samples were performed using standard methods adopted from the International Commission of Microbiological Specification on Foods (ICMSF) (Braide et al., 2011). A sample of 60 ml of sterile peptone water (0.1%, wt/vol) was added to 30 g of rice and homogenized for 1 min at 2000 rpm with a Lab-blender 400 stomacher (Laboratory Equipment, London, UK). All the samples were diluted decimally and adjusted to 10^4 of cells using a haemocytometer. An aliquot portion (0.1 ml) of each dilution was inoculated in triplicate onto the surface of solidified freshly prepared nutrient PDA. The plates were spread evenly with a sterile spreader and incubated for 3–5 days at 28 ± 2 °C.

2.8. In situ radiosensitization of A. niger and P. chrysogenum

Inoculated rice samples treated with basil EO were irradiated at 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0 kGy as described previously. D_{10}-

values were determined for *A. niger* and *P. chrysogenum* considering the kinetics of fungal destruction with and without basil EO and evaluated by linear regression considering the reciprocal of the slope of the line produced by plotting fungal counts (log CFU/g) against the irradiation doses. The microbial counts were normalized by applying a coefficient for both control and treatment. The D_{10} values for each fungus (*A. niger and P. chrysogenum*) were calculated in kGy based on Maity et al. (2011). After irradiation, samples were immediately incubated at 28 ± 2 °C. The relative sensitivity (RS) was calculated using the equation described by Caillet, Millette, Turgis, Salmieri, and Laroix (2005).

Relative radiation sensitivity(RS)

= (radiation D_{10} of control samples)/

The D_{10} is defined as the radiation dose required reducing the fungal population by 1 log or decreasing it by 90%.

2.9. Statistical analysis

The experimental design was composed of a 3 × 3 × 3 × 4 randomized complete block design with 3 replicates ($n = 3$), 3 days of analysis (days 1, 7, 14), 3 irradiation doses (0, 1, 2 kGy) and 4 concentrations of basil EO (0, 1, 2, 4% v/wt). The statistical processing was performed based on the Generalized Linear Model (GLM) univariate analyses using fungal count as the dependent variable, and the day, dose and concentration as the independent variables to evaluate the interaction effects between the variables i.e. day-radiation dose, day-EO concentration, radiation dose-EO concentration and day-radiation dose-EO concentration, for both *A. niger* and *P. chrysogenum*. Comparison of means between treatments was done on the effect of dose and concentration on each day of analysis by Duncan's multiple range tests at 5% level, and analysis of variance was performed using the PASW Statistics Base 18 software (SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Determination of MIC

The MIC of basil EO against *A. niger* and *P. chrysogenum* in the current study was 0.1% (v/v) basil EO, since no fungal colony growth was observed at this concentration. At lower concentrations (0.005% and 0.01%), fungal colonies started to appear after 8 h of incubation. A study conducted by Dube, Upadhyay, and Tripathi (1989) found that basil EO exhibited a wide fungitoxic spectrum capable of suppressing the mycelial growth of 22 species of fungi by poisoned food technique using Czapek-Dox agar medium for 7 days at 26 °C ± 2 °C. They showed that a concentration of 0.1% (v/v) basil EO could completely inhibit mycelial growth of *A. niger* and *P. chrysogenum*. Zollo et al. (1998) reported the MIC for 10^4 CFU/ml of *Aspergillus flavus* as 0.5% (v/v) by broth dilution micro method at 25 °C for 7 days of incubation period using Sarbouraud glucose broth. Basılico and Basılico (1999) studied the inhibitory effects of several essential oils, including basil EO, on the mycelial growth and ochratoxin A production by *Aspergillus ochraceus* using yeast-extract-sucrose broth at 25 °C and assessing fungal growth by drying and weighing mycelial mats; they reported that at a concentration of 1000 ppm basil EO was effective against 10^6 spore/ml of *A. ochraceus* up to 7 days. However, mold growth was detected after this incubation period. Although it may not be appropriate to compare MICs measured by different studies due to inherent variations in experimental parameters and conditions including sources of essential oils, fungal strains tested and assay protocols implemented (Jakowienko et al., 2011), our study advocates the potent mycotoxic properties of basil EO with an observed MIC as low as 0.1% (v/v) against both *A. niger* and *P. chrysogenum*.

3.2. In vitro fungal radiosensitivity in Potato Dextrose Broth (PDB)

Both *A. niger* and *P. chrysogenum* were found to be sensitive to irradiation and basil EO treatments. The D_{10} value for the controls (irradiation only) was 0.49 and 0.47 kGy for *A. niger* and *P. chrysogenum* respectively (Figs. 1 and 2). The D_{10} value was 0.43 and 0.31 kGy for 1 and 2% of basil EO, respectively, for *A. niger*. Similarly, the D_{10} value was 0.44 and 0.34 kGy for 1 and 2% of basil EO, respectively, for *P. chrysogenum*. Our findings corroborate similar studies with related species of fungi. Aziz, Moussa, and Ferial (2004) obtained a D_{10} value of 0.52 kGy for *A. flavus* isolated from spices. Blank and Corrigan (1995) reported D_{10} values for five different *Aspergillus* species ranging from 0.21 to 0.32 kGy, mentioning a D_{10} for *A. niger* of 0.245 kGy. They also reported the D_{10} values for six different *Penicillium* species ranges from 0.24 to 0.33 kGy. The fungal species for their experiment were obtained from research laboratories. Zeinab, Hala, Mohie, and Seham (2001) found a D_{10} value of 0.50 and 0.40 kGy for *P. chrysogenum* and *Penicillium crylophillum* respectively.

Our results showed that the D_{10} values decreased for both fungal species as the concentration of basil EO increased, resulting in an increase in RS. The RS increased from 1.14 to 1.55 for 1% and 2% basil EO for *A. niger*, and from 1.07 to 1.39 for 1% and 2% basil EO against *P. chrysogenum* as compared to the control. In the presence of 2% basil EO, no colony was observed after irradiation at 2 kGy for both fungi. These results demonstrated that basil EO can increase the RS of the tested fungal species. Although there are many studies on the relative sensitivity of pathogenic bacteria, similar studies with fungi are limited. Turgis, Borsa, Millette, Salmieri, and Lacroix (2008) found that the addition of EOs or their constituents to ground beef before irradiation reduced the radiation dose required to eliminate *Salmonella* Typhi. Chiasson, Borsa, Ouattara, and Lacroix (2004) reported that the addition of thyme EO or its main constituents to ground beef before irradiation increased the RS of *Escherichia coli* and *Salmonella* Typhi by up to 10 times. It is generally believed that the site of action of EOs is principally the cell cytoplasmic membrane of microorganisms. Oussalah, Caillet, and

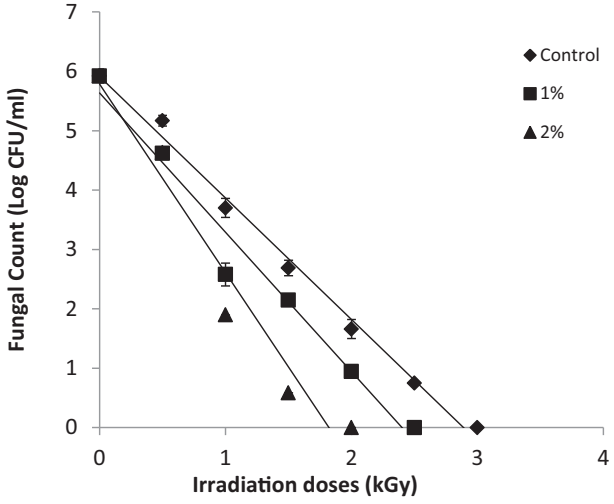

Fig. 1. Relative sensitivity of *A. niger* in the liquid medium with different concentrations (0–2%) of basil EO. Regression equations for plot are as follows: $y = -2.0457x + 5.91$ ($R^2 = 0.99$) for control, $y = -2.3459x + 5.6345$ ($R^2 = 0.97$) for 1% EO and $y = -3.164x + 6.4574$ ($R^2 = 0.9441$) for 2% EO. Relative sensitivity (RS) obtained for control experiment was 1, 1% EO was 1.14 and 2% EO was 1.55.

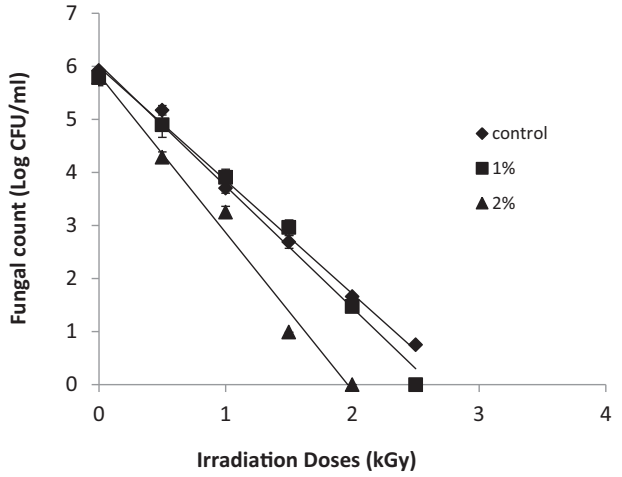

Fig. 2. Relative sensitivity (RS) of *P. chrysogenum* in liquid medium at different basil EO concentrations (0–2%). Equations for each regression line and RS are as follows: for control, $y = -2.1366x + 5.9857$ ($R^2 = 0.99$); for 1% EO, $y = -2.2954x + 6.0402$ ($R^2 = 0.98$); for 2%, $y = -3.0164x + 6.1711$ ($R^2 = 0.98$). Relative sensitivity (RS) obtained for control experiment was 1, 1% EO was 1.07 and 2% EO was 1.39.

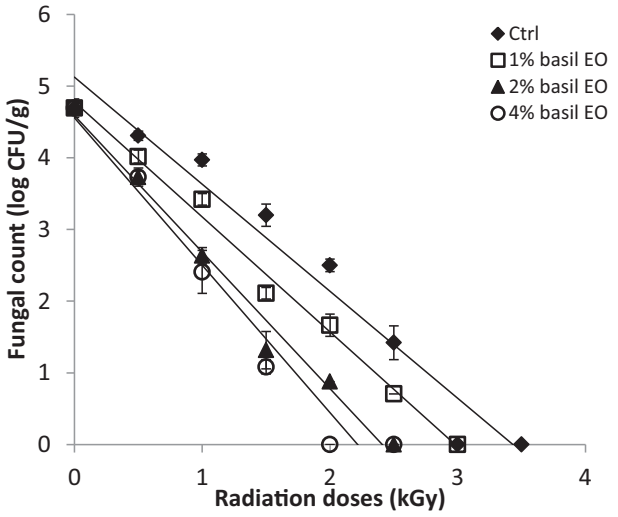

Fig. 3. Relative sensitivity (RS) of *A. niger* at different basil EO concentrations (0–4% v/wt) on rice grain. Equations for each regression line and RS are as follows: for Ctrl, $y = -1.4943x + 5.1268$ ($R^2 = 0.98$); for 1% EO, $y = -1.6045x + 4.7806$ ($R^2 = 0.99$); for 2%, $y = -1.9057x + 4.5914$ ($R^2 = 0.98$); for 4% EO, $y = -2.0566x + 4.45568$ ($R^2 = 0.96$). Relative sensitivity (RS) obtained for control experiment was 1, 1% EO was 1.07, 2% EO was 1.27 and 4% EO was 1.37.

Lacroix (2006) showed that EOs derived from Spanish oregano (*Corydothymus capitatus*), Chinese cinnamon (*Cinnamomum cassia*), and savory (*Satureja montana*) affect the membrane integrity of bacteria and induce a depletion of intracellular ATP concentration. They also showed, through electronic microscopy, that the EOs induces significant damage to the bacterial cytoplasmic membrane. In addition, ionizing radiation initiates a series of events that impairs the cell structure which is further compounded by the addition of EO leading to the disintegration of the cytoplasmic membrane, making it impossible for the cell to repair the damage incurred by the complementary action of both treatments (Chiasson, Borsa, Ouattara, & Lacroix, 2004; Takala, Salmieri, Vu, & Lacroix, 2011; Turgis et al., 2008). Hence the combined treatment showed greater efficiency against the tested fungi than the individual treatment of basil EO or irradiation alone.

3.3. *In situ* relative sensitivity of *A. niger* and *P. chrysogenum*

The D_{10} values for controls (irradiation only) were 0.67 and 0.63 kGy for *A. niger* and *P. chrysogenum* respectively. The D_{10} value decreased significantly ($P \leq 0.05$) for both fungi with the addition of 4% basil EO. For *A. niger*, D_{10} values were 0.62, 0.52, and 0.49 kGy for 1, 2 and 4% of basil EO respectively, and the RS increased 1.07, 1.27 and 1.37 times respectively as compared to the control (Fig. 3). For *P. chrysogenum*, D_{10} values were 0.46, 0.41 and 0.39 kGy for 1, 2 and 4% of basil EO respectively. A similar trend was observed with *P. chrysogenum* where the RS increased with increasing basil EO concentrations with respective values of 1.36, 1.50 and 1.60 as compared to the control (Fig. 4). The D_{10} values in inoculated rice grain were higher than the D_{10} values obtained in the *in vitro* in the PDB medium. Such difference in D_{10} values for the *in situ* and *in vitro* studies may be explained by the phase states of the basil EO. In the *in situ* experiment, basil EO diffused as vapor from the sponge to reduce fungal growth in the rice grains, whereas in the *in vitro* study basil EO was directly incorporated in the medium which provided greater surface area for the EO to induce antifungal activity by direct contact. Such observations also may be due to a concentration effect of the EO. In the *in vitro* experimental condition, the EO may not be subjected to major concentration variation while in the *in situ* condition the EO diffusion may result in the dilution of the EO molecules by air thereby reducing the toxicity of the active components. It has been reported that EOs are more active in direct contact methods as the active components can directly inhibit the metabolism of microbial cells (Ikeura, Somsak, Kobayashi, Kanlayanarat, & Hayata, 2011). Lopez et al. (2005) also showed that inhibition in solid diffusion tests was stronger than in vapor tests. This observation is also well explored in another study conducted by Dobre, Gagiu, and Niculita (2011) who evaluated the *in vitro* antimicrobial activity of seven EOs against four different bacterial and five fungal strains that are involved in food poisoning using agar disc diffusion and disc volatilization methods. Using 10 μl basil EO they could observe antimicrobial and antifungal activities against all the tested bacteria and fungi using the diffusion

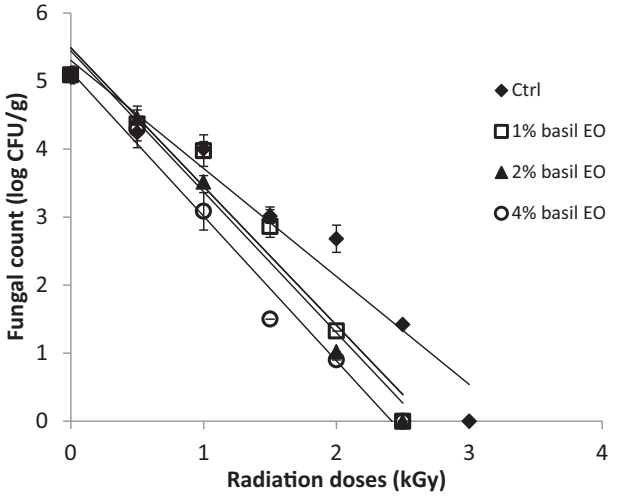

Fig. 4. Relative sensitivity (RS) of *P. chrysogenum* at different basil EO concentrations (0–4% v/wt) on rice grain. Equations for each regression line and RS are as follows: for Ctrl, $y = -1.5879x + 5.3018$ ($R^2 = 0.96$); for 1% EO, $y = -2.0396x + 5.4869$ ($R^2 = 0.96$); for 2%, $y = -2.071x + 5.4404$ ($R^2 = 0.96$); for 4% EO, $y = -2.1272x + 5.138$ ($R^2 = 0.99$). Relative sensitivity (RS) obtained for control experiment was 1, 1% EO was 1.36, 2% EO was 1.50 and 4% EO was 1.60.

method; however, no such activity could be observed using the vapor method.

In the *in situ* study, the D_{10} values for the control were higher as compared to the *in vitro* D_{10} values against *A. niger* and *P. chrysogenum* respectively. *In situ* experiments with basil EO at a concentration of 4% had D_{10} values of 0.49 kGy against *A. niger* and 0.39 kGy against *P. chrysogenum* whereas the D_{10} values with 2% basil EO in the *in vitro* studies (0.31, 0.34 kGy respectively). This observation may result from the fact that food components exhibit a protective nature and cause D_{10} values to be always higher as compared to culture media. According to Burt (2004), a greater concentration of EO is required in case of food system to have the same efficiency as the *in vitro* assay. In addition, it may also be explained by the instability of some of the basil EO components such as limonene and α-pinene in the vapor phase as compared to their stable state conditions in the aqueous phase. In the vapor phase, it has been shown that these components cause rapid gas phase reactions with atmospheric oxidants to yield less toxic oxygenated products (Dorman & Deans, 2000; Ikeura et al., 2011).

3.4. Combined effect of irradiation and basil EO on inoculated rice grains during storage

The antifungal effects of basil EO vapor on the growth of *A. niger* and *P. chrysogenum* on rice grains during up to 14 days of storage at 28 °C ± 2° are presented in Tables 1 and 2. The fungal density was highly significant ($p \leq 0.01$) for the effects of day, dose and concentration and for all interactions effects. Therefore, means separations were done on the effect of dose and concentration on each day the tests were evaluated (1, 7 and 14 days). All samples had an initial fungal load of 4 log CFU/g of rice grains at day 0.

For *A. niger* at day 1, no significant difference ($p \leq 0.05$) was observed between control and 1% of basil EO. Irradiating the samples with 2 kGy of radiation dose alone resulted in 2.51 log CFU/g of fungal growth representing a significant ($p \leq 0.05$) reduction of 2.18 log CFU/g as compared to the control. On the other hand, applying 2% EO without irradiation resulted in a fungal count of 4.27 log CFU/g thereby showing a decrease of 0.42 log CFU/g. At day 7, the presence of 2% EO without any radiation led to the formation of 4.10 log CFU/g of fungus which corresponded to a 0.59 log CFU/g reduction as compared to the control of day 1. This also represents a significant ($p \leq 0.05$) reduction of 1.64 log CFU/g as compared to 5.74 log CFU/g obtained in the absence of EO and radiation altogether at day 7. At day 14, the addition of 2% without any radiation caused a significant ($p \leq 0.05$) reduction of 1.18 log CFU/g as compared to the control (4.69 log CFU/g), and a significant ($p \leq 0.05$) decrease of 2.01 log CFU/g as compared to 5.52 log CFU/g obtained in the control of day 14. Considering the whole duration of the study, the addition of 2% EO caused a reduction in fungal count by 0.4 log CFU/g on day 1 to 1.18 log CFU/g on day 14. The application of 2 kGy of radiation alone led to a reduction of 1 log CFU/g in fungal count from day 1 to day 14. Combining with 2% basil EO with 2 kGy irradiation dose reduced significantly ($p \leq 0.05$) 5 log CFU/g at day 14 as compared to the control. This study demonstrates the efficacy of basil EO in combination with irradiation in reducing fungal growth. Previous studies have investigated the individual application of either plant-derived EOs (Aldred, Cairns, & Magan, 2008; Gibriel, Hamza, Gibriel, & Mohsen, 2011; Sumalan, Alexa, & Poiana, 2013) or radiation separately (Gibriel, Mohsen, & Hamza, 2009; Menasherov, Paster, & Nitzan, 1992) against various fungal species. However, our study purports the enhanced inhibitory effect that can be achieved by combining EO and ionizing radiation treatment against fungal microorganisms. Accrued inhibitory effects were observed on all the samples tested against fungal growth from day 1 using the combined treatment. At day 7 a dose of 2 kGy of radiation without any basil EO led to a decrease in 0.93 log CFU/g as compared to the control of day 1. However, combining the 2% basil EO treatment with 2 kGy of radiation resulted in the complete absence of fungal growth at day 7 and 14. Higher inhibitory activity was observed with 4% basil oil.

For *P. chrysogenum*, similar trends were observed following treatment with EO or irradiation alone or in combination. At day 1, in the presence of 2% EO alone, a reduction of 0.76 log CFU/g of fungus was observed as compared to the control. On the other hand, irradiating the samples with 2 kGy dose individually led to a decrease of 2.41 log CFU/g in fungal colonies. Combining the 2% EO and a radiation level of 2 kGy resulted in a decrease of 4.09 log CFU/g. At day 7, treatment with 2% EO alone led to a reduction of 1.84 log CFU/g while an irradiation treatment alone caused a decrease of 2.61 log CFU/g of fungus as compared to the day 1 control (5.09 log CFU/g). Applying a 2% EO with a radiation level of 2 kGy in combination completely inhibited the fungal growth. At day 14, a reduction of 1.32 log CFU/g was observed following a 2% EO treatment alone which is significant ($p \leq 0.05$) while a 2.84 log CFU/g reduction was obtained with 2 kGy radiation treatment only. Combining a 2% EO treatment with a 2 kGy radiation level caused a complete inhibition of fungal growth.

In the present study the complete inhibition of fungal growth was observed at 2.5 kGy radiation doses for *A. niger* and *P. chrysogenum*. Treating with essential oil led to a lower radiation

Table 1
Fungal count (CFU/g) on rice grain inoculated with *A. niger* (10^4 conidia/g) treated with basil EO (0, 1, 2, 4% v/wt) and 0, 1 and 2 kGy during storage at 1, 7 and 14 days.

Day	Irradiation (kGy)	Concentration of basil oil (% v/wt)			
		0	1	2	4
1	0	4.69 ± 0.08^{cC}	4.64 ± 0.04^{cC}	4.27 ± 0.67^{cB}	3.91 ± 0.61^{cA}
	1	3.97 ± 0.12^{bD}	3.39 ± 0.08^{bC}	2.39 ± 0.13^{bB}	2.09 ± 0.18^{bA}
	2	2.51 ± 0.10^{aD}	1.50 ± 0.0^{aC}	0.75 ± 0.10^{aB}	ND^{aA}
7	0	5.74 ± 0.05^{cD}	5.53 ± 0.05^{cC}	4.10 ± 0.08^{cB}	3.89 ± 0.03^{cA}
	1	4.28 ± 0.06^{bC}	4.23 ± 0.07^{bC}	3.50 ± 0.29^{bB}	2.04 ± 0.2^{bA}
	2	3.76 ± 0.08^{aC}	1.57 ± 0.98^{aB}	ND^{aA}	ND^{aA}
14	0	5.52 ± 0.31^{cD}	4.55 ± 0.10^{cC}	3.51 ± 0.06^{cB}	2.93 ± 0.16^{cA}
	1	3.97 ± 0.10^{bC}	3.79 ± 0.1^{bC}	3.08 ± 0.05^{bB}	1.63 ± 0.36^{bA}
	2	3.58 ± 0.06^{aC}	3.30 ± 0.46^{aB}	ND^{aA}	ND^{aA}

Values are means ± standard deviations. Within each row, means with the same uppercase letter are not significantly different ($P > 0.05$). Within each column and each day of analysis, means with the same lowercase letter are not significantly different ($P > 0.05$).
ND corresponds to non-detectable.

Table 2
Fungal count (log CFU/g) on rice grain inoculated with *P. chrysogenum* (10^4 conidia/g) treated with basil EO (0, 1, 2, 4% v/wt) and 0, 1 and 2 kGy during storage at 1, 7 and 14 days.

Day	Irradiation (kGy)	Concentration of basil oil (% v/wt)			
		0	1	2	4
1	0	5.09 ± 0.13^{cD}	4.40 ± 0.06^{cB}	4.33 ± 0.08^{cAB}	4.24 ± 0.04^{cA}
	1	4.00 ± 0.611^{bD}	3.75 ± 0.20^{bC}	3.04 ± 0.08^{bB}	2.57 ± 0.23^{bA}
	2	2.68 ± 0.45^{aB}	1.25 ± 0.36^{aA}	1.00 ± 0.01^{aA}	0.75 ± 0^{aA}
7	0	4.28 ± 0.07^{bA}	4.07 ± 0.08^{bC}	3.25 ± 0.08^{cB}	3.10 ± 0.07^{cA}
	1	3.88 ± 0.05^{bD}	3.67 ± 0.12^{bC}	2.67 ± 0.1^{bB}	2.46 ± 0.11^{bA}
	2	2.48 ± 0.54^{aC}	1.25 ± 0.36^{aB}	ND^{aB}	ND^{aA}
14	0	4.45 ± 0.51^{cD}	3.99 ± 0.06^{cC}	3.77 ± 0.24^{cB}	3.22 ± 0.04^{cA}
	1	3.94 ± 0.07^{bD}	3.08 ± 0.05^{bC}	2.94 ± 0.06^{bB}	2.26 ± 0.18^{bA}
	2	2.25 ± 0.20^{aB}	1.32 ± 0.39^{aB}	ND^{aA}	ND^{aA}

Values are means ± standard deviations. Within each row, means with the same uppercase letter are not significantly different ($P > 0.05$). Within each column and each day of analysis, means with the same uppercase letter are not significantly different ($P > 0.05$).
ND corresponds to non-detectable.

dose for the complete inhibition of the fungal growth. Menasherov et al. (1992) reported that *A. flavus* and *A. ochraceus* isolated from different cereal stop germination at 2.5 kGy. Similarly, Gibriel et al. (2009) found that γ-radiation at a dose of 3 kGy was quite efficient to stop the growth of *A. flavus* in stored corn and aflatoxin production. In another study, Aziz, El-Fouly, Abu-Shady, & Moussa (1997) reported that the dose required for complete inhibition of fungi in different food and feed products ranged from 4 to 6 kGy.

However, all the above studies were conducted using either EO or irradiation as the inhibitory source for fungal growth. Our study shows that supplementing irradiation treatment greatly enhances the efficiency of basil EO. The combined treatment of basil EO and ionizing radiation was more efficient against fungal growth than that of individual treatment with either basil EO or gamma radiation alone. These results suggest that basil EO can be used to increase the relative sensitivity of *A. niger* and *P. chrysogenum* to irradiation treatment. It has also been well established that irradiation in combination with other treatments suppress the growth of surviving microorganisms in vegetables, meat products during storage (Caillet et al., 2005; Mostafavi, Mirmajlessi, & Fathollahi, 2012; Thayer & Boyd, 1999). The present study showed the antifungal efficacy of basil EO in combination with irradiation in rice grains and represents a potential approach that could be applied commercially to protect stored grain product.

4. Conclusion

This study showed the enhanced antifungal effects that can be achieved by combining a treatment of basil EO and gamma radiation against *A. niger* and *P. chrysogenum*. *In situ* studies carried out showed that the basil EO in conjunction with the ionizing radiation can be effectively used to control fungal growth in rice. Moreover, our data support the fact that a combined treatment of ionizing radiation and basil EO can significantly increase the relative sensitivity of fungal species and result in a more pronounced inhibition of fungal growth as compared to individual treatments. The combined ionizing radiation-basil EO treatment, thus, offers a promising approach to control food contamination by fungi in ambient storage conditions. Further research may be warranted to gain understanding on the mode of action of a combined EO-irradiation treatment in extending the shelf life of stored products.

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