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IRRADIATION EFFECT ON ANTIFUNGAL POTENTIAL *Myristica fragrans* (NUTMEG) ESSENTIAL OIL, A PRELIMINARY STUDY

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ABSTRACT

The *Myristica fragrans* (nutmeg) is any of several species of trees in genus Myristica. The most important commercial species is Myristica fragrans, an evergreen tree indigenous to the Banda Islands in the Moluccas of Indonesia, or Spice Islands. Widely used as a food condiment and flavoring, used in perfumery and medicine. The nutmeg has many biological properties as analgesic, antiseptic, antispasmodic, aphrodisiac, astringent, carminative, haemostatic, insecticidal and parasiticide. Previous research has revealed interesting antimicrobial effect of nutmeg essential oil. Spices irradiation is a worldwide process used and this technique is an effective pathogenic microorganisms control providing consumers food security. By the fact nutmeg not only used in food, but also as an essential oil raw material this study investigated the nutmeg different irradiation doses influence on the possible antimicrobial potential oil. The aim of this study was evaluate the antifungal potential oil from unirradiated and irradiated nutmeg in the fungus *Guinardia citricarpa* that causes serious damage in orange plantations. The *Myristica fragrans* samples were irradiated in ^{60}Co irradiator at doses of 5.0 and 10.0 kGy. The oil was used in 50 and 100% of concentration (irradiated and not irradiated). The essential oil in high concentration could be a good substitute for the pesticides used to control the fungus Guignardia citricarpa, the nutmeg irradiation treatment with the doses 5.0 and 10.0 kGy did not changed the results in the essential oil effectiveness. It is clear that is necessary others studies with others doses to verify the radiation ionizing influence in the essential oil activity and the oil application efficiency in the field against the fungus.

1. INTRODUCTION

There has been an increasing consumer demand for foods free or with low added synthetic preservatives that could be toxic [1]. Concomitantly, consumers have also demanded for wholesome and safe food with long shelf lives. These requirements press on the food industry for progressive chemical preservatives removal and adoption of natural alternatives to obtain safe food with long shelf lives [2]. Some plants demonstrate antimicrobial activity [3] with application in the food industry as antibacterial and antifungal agents [4]. The spices can be used safety of synthetic preservatives [5]. Although the primary purpose of spices is to impart food flavor, the antimicrobial and antioxidant properties have also been exploited [6]. The antimicrobial activity was documented and the interest continues to the present [7; 8] by the increase of fungal resistance to classical drugs and the treatment costs. In spite the introduction of new antifungal drugs, they are limited in number, so aromatic plants have been widely used in folk medicine. It is known that most of their properties are due to their volatile oils. Essential oils from many plants are known to the antifungal activity [9]. The nutmeg (*Myristica fragrans*) is any of several species of trees in genus *Myristica*. The most important commercial species is *Myristica fragrans*, an evergreen tree indigenous to the Banda Islands in the Moluccas of Indonesia, or Spice Islands. Widely used as a food

condiment and flavoring, used in perfumery and medicine. The nutmeg has many biological properties as analgesic, antiseptic, antispasmodic, aphrodisiac, astringent, carminative, haemostatic, insecticidal and parasiticide. Previous research has revealed interesting antimicrobial effect of nutmeg essential oil. Miristic acid, glycerides, stearic, lauric, linoleic, palmític. The essential oil is traditionally obtained by hydrodistillation, steam distillation or solvent extraction [10]. The application of ionizing radiation in food is based mainly on the fact that ionizing radiation damage very effectively the DNA and living cells become inactivated. Are three the irradiation doses: “Low” doses (up to 1 kGy), “Medium" doses - (1-10 kGy) and "High" doses - (greater than 10 kGy) [11]. The insects, microorganisms and plant are prevented from reproducing, resulting in preservative effects. At the same time other chemical changes in food are minimal [12]. By the fact the ionizing radiation is widely used in spices decontamination, the aim of this study was to investigate the potential antifungal of nutmeg oil in the concentration of 50 and 100% and irradiated at doses level of 5.0 and 10.0 kGy to verify the influence in the nutmeg essential oil against the fungus *Guignardia citricarpa*. The Citrus black spot (CBS) caused by *Guignardia citricarpa* has become a serious widespread problem for citrus production [13]. Develop on the fruit [14] resulting in yield loss. Due to the presence of CBS in many countries in the Southern Hemisphere, the European Community (EC) and the United States (U.S.) severely limit importation of fresh citrus fruit from those countries where the disease is present [15]. Thus, fruit from countries with CBS can not be exported [16].

2. MATERIALS AND METHODS

2.1. Samples

The nutmeg (100g per sample) was purchased from Hikari Industria e Comércio LTDA – BRAZIL.

2.2. Irradiation

The nutmeg samples were irradiated in polyethylene packaging at IPEN-CNEN/SP, at dose level of 0, 5.0 and 10.0 kGy using a ^{60}Co gamma ray facility (Gammacell 220, A.E.C.L., dose rate: 1.73 kGy/h) Harwell Amber 3042. Dosimeters were used for the measurement of radiation dose.

2.3. Hydrodistillation

The essential oils (EO) were extracted by the hydrodistillation method [17]. The oil was used in the concentration of 50 and 100%.

2.4 Fungal Isolation and Identification

The fungal (*Guignardia citricarpa*) isolation and identification [18].

2.5 Antifungal test preparation

Four small fungus (*Guignardia citricarpa*) portions were placed in each petri dish containing agar (potato dextrose Agar) and a drop of essential oil irradiated and not irradiated with 50

and 100% of concentration was placed on each fungus portion. The test was done with three samples in triplicate, totalizing nine repetitions. The samples were incubated for ten days. The oil effectiveness was done verifying the fungus growth or not.

3. RESULTS AND DISCUSSION

The nutmeg essential oil did not showed effectiveness antifungal property with the oil 50% not irradiated and irradiated with 5.0 kGy (Fig. 1) as expected and reported by some authors with others spices oils [19-21]. The same happens with the nutmeg oil irradiated with 10.0 kGy (Fig. 2).

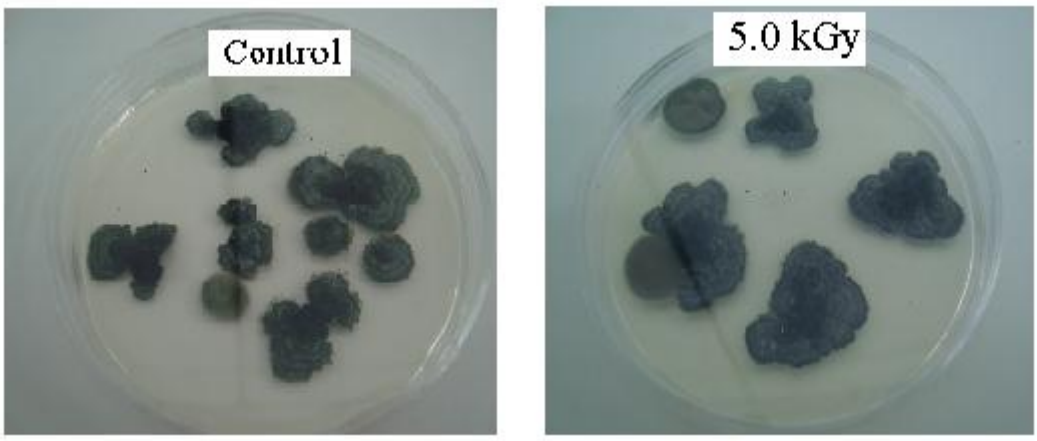

Figure 1. Fungus (*Guignardia citricarpa*) growth in samples inoculated with nutmeg oil not irradiated and irradiated with 5.0 kGy and 50% of concentration.

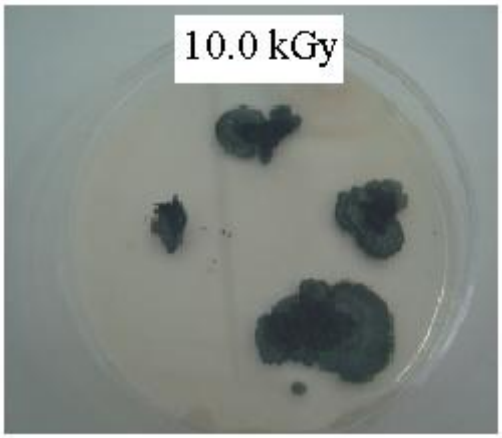

Figure 2. Fungus (*Guignardia citricarpa*) growth in samples inoculated with nutmeg oil irradiated with 10.0 kGy and 50% of concentration.

The literature has recorded the extracts efficiency from a wide range of species to promote inhibition of several fungal phytopathogens [22; 23]. As expect, in the in vitro experiment, the fungal (*Guignardia citricarpa*) growth was higher in the control sample (Fig. 3) than the sample with nutmeg essential oil not irradiated (control) and irradiated at dose levels of 5.0 and 10.0 kGy (Fig. 1 and 2).

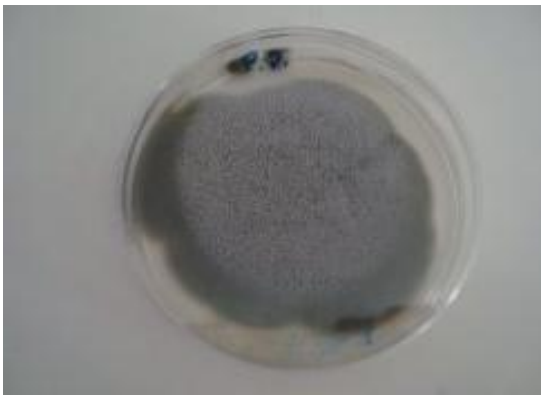

Figure 3. Fungus growth control sample.

In the second day of incubation, the samples with nutmeg oil not irradiated about the concentration of 50% already showed signs of fungal activity, this sings was like drops of water (Fig. 4) [24].

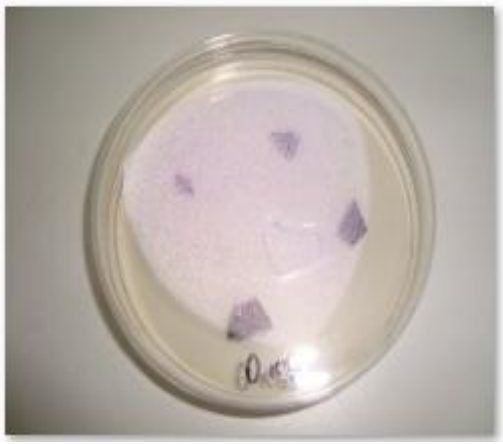

Figure 4. Two days of incubation, sings of fungus activity. Oil not irradiated and with 50% of concentration.

As figure 4, in the second day of incubation the samples of concentration 50% and irradiated with 5.0 and 10.0 kGy showed signs of fungal activity (Fig. 5) [24].

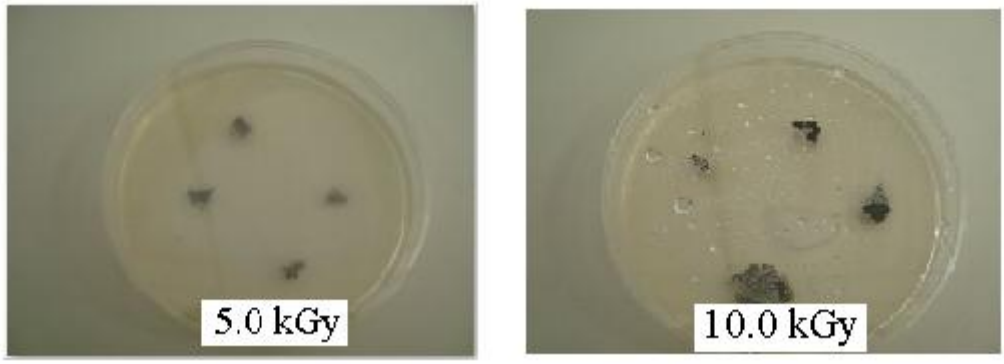

Figure 5. Two days of incubation, sings of fungus activity. Oil irradiated with 5.0 and 10.0 kGy with 50% of concentration.

Analyzing the samples (Fig. 4 and 5) was possible to verify that the fungus growth was lower in the samples with oil when compared with control samples (Fig. 3). The oil effectiveness in low concentration was not so high [25].

Already in the samples of concentration of 100%, not irradiated and irradiated with 5.0 and 10.0 kGy (Fig. 6), in the second day the sings of activity was not verified like in the samples with oil of 50% (Fig. 4 and 5) [25 and 26].

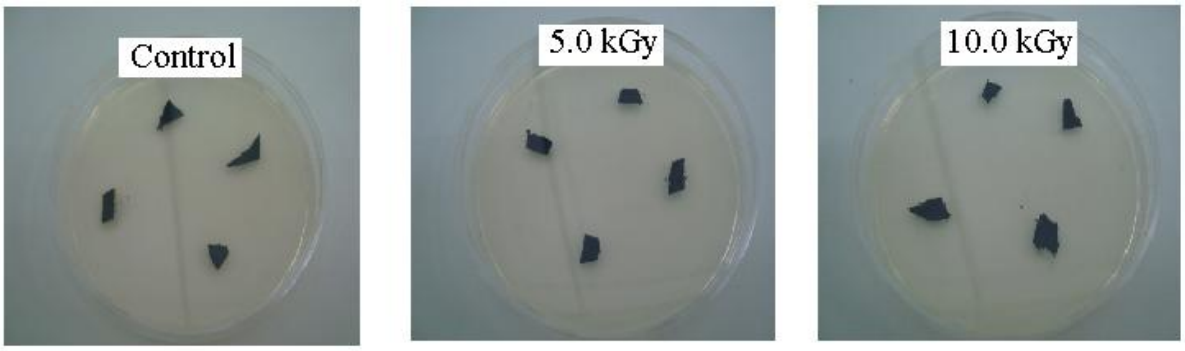

Figure 6. Two days of incubation, without sings of fungus activity. Oil not irradiated (control) and irradiated with 5.0 and 10.0 kGy in the concentration of 100%.

With 10 days of incubation the samples with oil 100%, not irradiated and irradiated with 5.0 and 10.0 kGy the fungus growth did not occur (Fig. 7). The fungus was inactivated for some days, this result is similar to other result obtained [25].

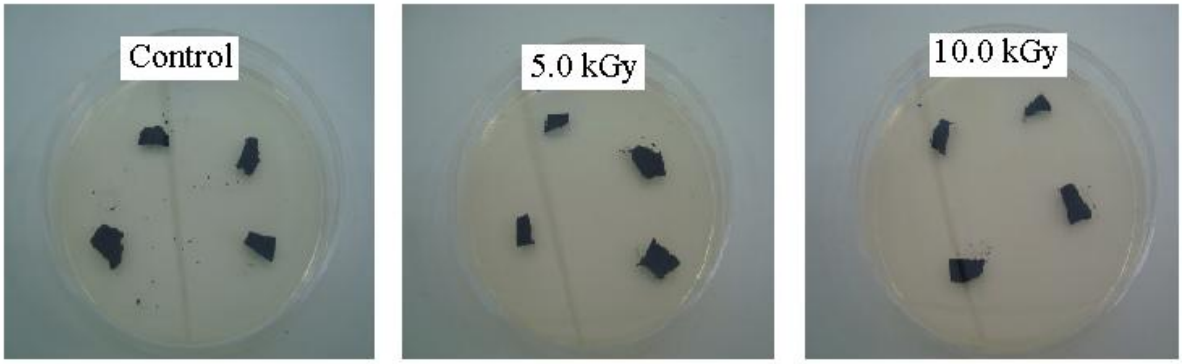

Figure 7. Sample with oil not irradiated (control) and irradiated with 5.0 and 10.0 kGy in the concentration of 100%. Inactivation of the fungus Guignardia citricarpa for 10 days.

This result may be related to the action of nutmeg compounds. In lower concentrations the oil was not efficient like some others oils, only with the 100% concentration the oil inhibited the fungus growth for some days (Fig. 7), in other study the nutmeg oil in the concentration of 77% was capable to control various pathogenic fungi and bacteria [26] .

4. CONCLUSIONS

The essential oil in high concentration could be a good substitute for the pesticides used to control the fungus *Guignardia citricarpa*, the nutmeg irradiation treatment with the doses of 5.0 and 10.0 kGy did not changed the results in the essential oil effectiveness. It is clear that is necessary others studies with different doses and more days to verify the radiation ionizing influence in the essential oil activity and the oil application efficiency in the field against the fungus. Is necessary others studies with humans and environment toxic effects and negative interactions, so that we can recommend the use in commercial-scale.

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