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Effect of Propolis and Clotrimazole on Controlling Aflatoxin in Pistachio (*Pistacia vera* L.)

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ABSTRACT

In order to determine the effect of propolis on the growth of *Aspergillus flavus* and *Aspergillus parasiticus*, the most important fungi producing aflatoxin in pistachio nuts, some solvents e.g. hexane, dichloromethane, methanol and warm water were used for extraction and finally five extract powders of propolis were obtained. The effect of extract powders dissolved in DMSO solvent on the growth of mentioned fungi were investigated on special CMA culture media. Statistical analysis showed that dichloromethane and hexane extracts had the most antifungal effects. Furthermore, the effect of dichloromethane extract for controlling aflatoxin was studied. Results showed that Akbari pistachio treated with propolis extracts had lower aflatoxin concentration than critical level. Pistachios treated with 12.5 mg mL^{-1} of T_B, which was the part of propolis dissolved in dichloromethane had significant difference with control ($p < 0.05$). Total aflatoxin levels were 1.28 ppb and 7.3 ppb for treatment and control, respectively.

Key Words: Propolis extract; Antifungal; Aflatoxin concentration; Pistachio nut

INTRODUCTION

Although honey is perhaps the most famous bee product of interest to human beings, bees also make propolis, another substance that humans have used for ages. Bees coat the hive with propolis. It provides protection against harmful fungi, bacteria and viruses. It is a resinous compound made primarily from tree sap. Propolis posses a pleasant aromatic smell and varies in color depending on its source and age. The composition of the propolis depends on the place and time of collection. As a consequence, more than 160 constituents have been identified so far among, which phenolic compounds including flavonoids, are major constituents (more than 50% of the propolis weight) (Banskota *et al*., 1998). Budrock *et al.* (1998) also identified the main classes present in propolis as flavonoids, phenolics and various aromatic compounds. Flavonoids are well-known plant compounds that have antibacterial, antifungal and antiviral characteristics (Grange & Davey, 1990) and are biologically active compounds, which come from its plant source. Propolis extracts are also great scavengers against free radicals and reactive oxygen species (ROS) and this is one of the reasons for health benefits (Pascual *et al*., 1994). Many agricultural commodities are vulnerable to attack by a group of fungi that are able to produce toxic metabolites called mycotoxins. Among various mycotoxins, aflatoxins have assumed significance due to their deleterious effects on human beings. It is a secondary metabolite and poisonous carcinogenic by products during the growth of several species of the mold fungus *Aspergillus* especially *Aspergillus flavus* Link and *A. parasitius* Speare, which contaminating many nuts. The major types of aflatoxins are B_1, B_2, G_1, G_2 and M_1. Among them aflatoxin B_1 is a very potent carcinogen to humans and animals (Mahoney & Rodriguez, 1996; Shahidi, 2004). Pistachio nuts are also sensitive to *Aspergillus* species. Mojtahedi *et al.* (1979) isolated 13 species of *Aspergillus* from pistachio kernels. Presence of aflatoxin concentration in kernels is an important criterion for export and universal competition. Wrong management of pistachio orchards during harvest and post harvest increases aflatoxin concentration in splitting nuts (Doster & Michailides, 1994). Due to the antifungal characteristic of propolis, its effect on prevention and control the production of aflatoxin in splitting pistachio nuts was determined.

MATERIALS AND METHODS

In order to study the effect of propolis (collected from Jiroft, Iran), five extractions were prepared by using four solvents e.g. hexane, dichloromethane, methanol and warm water. 10 mL of solvent was added to 10 mg of propolis. During the three days of protection time, it was continuously mixed by vortexing. After centrifugation at 2000 rpm (5 min), two phases were separated from the solution, supernatant and pellet. The pellet was dissolved in other solvent and these stages continued till the latter solvent. The supernatant was evaporated and the residue

used as treatment. Treatment A (T_A) was the part of propolis, which dissolved in hexane. T_B in dichloromethane; T_C in methanol; T_D in warm water and finally T_E was the part of propolis that did not dissolve in any solvent. Different concentrations including T_1, 1.56; T_2, 3.12; T_3, 6.25; T_4, 12.5; T_5, 25 and T_6, 50 mg mL^{-1} of each treatment were obtained by using dimethyl sulfoxide (DMSO) + methanol; 1:1 (v/v). DMSO with solvent was probed as control and clotrimazole fungicide (1/1000, v/v) was also evaluated. Pure fungus of *Aspergillus flavus* and *A. parasiticus* were cultured on CMA medium. After four days, the maximum inhibitory zone of each disk was observed and its diameter was recorded. In the other experiment, the best propolis extracts were used for *Pistacia vera* cv. Akbari using completely randomized design (CRD) with three replicates and analysis of aflatoxin levels of samples were measured by HPLC (Thompson Instrument Co., 2002).

RESULTS AND DISCUSSION

Results showed that T_B, T_A, T_C and T_D were effective on controlling the growth of fungal mycelia. T_E had no effect on inhibitory growth of fungal mycelia. The curves showed the minimum inhibitory concentration (MIC) controlling the growth of fungi. In order to determine MIC and showing the effect of different concentrations of each treatment, the charts of inhibitory zone range were compared (Fig. 1 - 5).

T_B with 12.5 mg mL^{-1} was applied for selected Akbari pistachio, which was a sensitive cultivar to Aspergillus species. Analysis of aflatoxin by individual methods of HPLC instrument showed that pistachio treated with 12.5 mg mL^{-1} of T_B had significant difference with control. Total aflatoxin levels were 1.28 ppb and 7.3 ppb for treatment and control, respectively ($p < 0.05$). This result showed that propolis can control aflatoxin in pistachio approximately by 82.5% and it is found that the effect of propolis was 3 fold compared to clotrimazole fungicide. The flavonoids in propolis extracts may be responsible for antifungal effects (Shub *et al*., 1978). The European Union applies some of the restrict regulations in the world on acceptable aflatoxin levels, i.e. 4 ppb as opposed to 10 - 15 ppb. In this study, treatment with propolis reduced the production of aflatoxins (total aflatoxins) to acceptable level (1.28 ppb). Accurate scientific programs of orchard management are the most important way of controlling aflatoxin before or during harvest and it is also clear that having pistachio nuts out of any harmful fungicides is so important, which is now a days common for controlling aflatoxin. Recently, propolis has also been extensively used in food and beverages to improve health and prevent diseases such as inflammation, heart disease, diabetes and even cancer (Bankova *et al*., 1996). Because of its wide range of biological activities and its use as a health food, we were confident of applying its extract on pistachio nuts. Application of propolis for controlling aflatoxin could be an acceptable substitution instead of fungicides.

Fig. 1. Part of propolis dissolved in dicloromethane

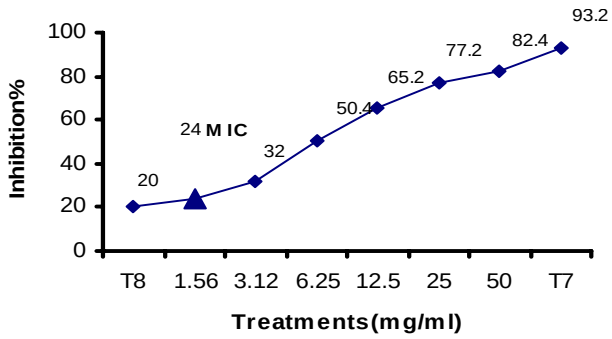


Fig. 2. Part of propolis dissolved in hexane

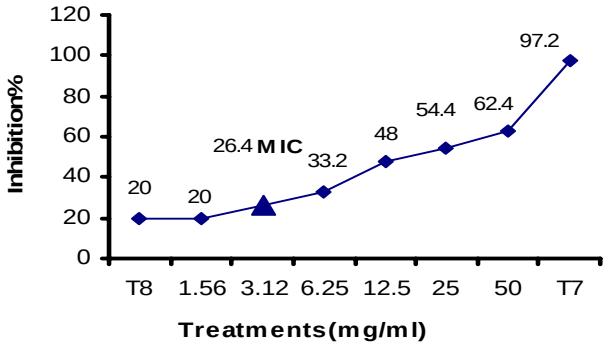


Fig. 3. Part of propolis dissolved in warm water

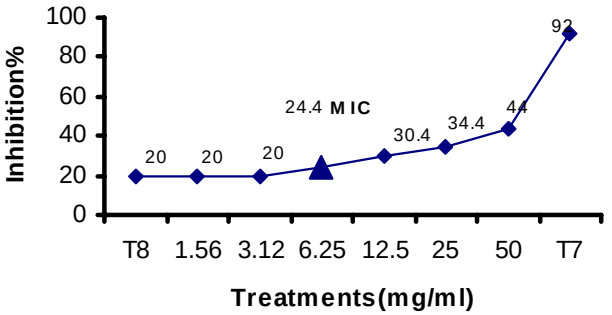


Fig. 4. Part of propolis dissolved in methanol

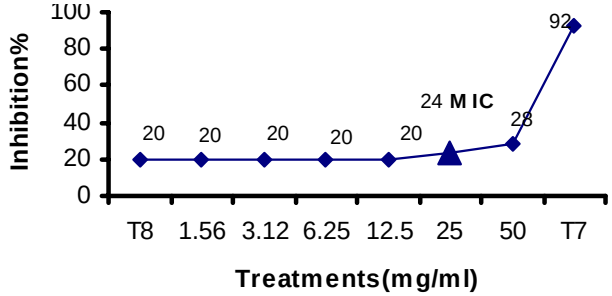
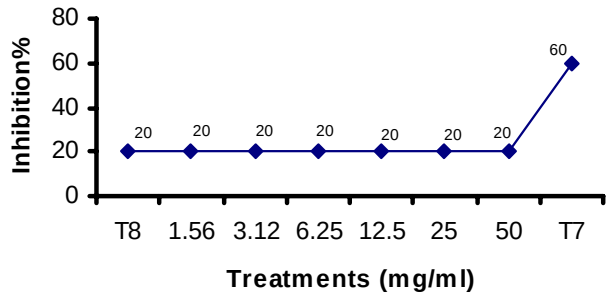


Fig. 5. Part of propolis not dissolved in any solvent



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